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MOTIVES FOR MURDER

FRANCIS E. CAMPS*

For the title of this paper, I wish to offer due acknowledgment to Miss Tennyson Jesse (1). She died in 1957 and I should like to offer it as a tribute to her memory.

Her name can be joined with those of Edmund Pearson (2, 3) and William Roughead (4, 5) as being the leading writers on Criminology during the last half century. Tennyson Jesse whom I had the privilege to meet first when she was preparing "The Case of Christie and Evans" (6) for the "Notable British Trial Series," was a writer with a remarkable skill in assessing the character of criminals upon whose weaknesses she could, in most cases, unerringly put a finger. She had an encyclopaedic knowledge of cases in the past and of weaknesses of the famous criminals.

To this must be added an immense sense of humour which she shared with Edmund Pearson of whom she was a great admirer. Two of the details associated with the Borden case (2) that were a constant amusement to her were the mutton soup for breakfast on the fatal day and the following doggerel—

> "Lizzie Borden took an axe and gave her Mother
> forty whacks, when she saw what she had done
> she gave her Father forty-one."

Her liking for this she shared, it is alleged, with President Roosevelt.

Tennyson Jesse considered that there were six primary motives for murder—

for Gain	for Jealousy
for Revenge	for Lust of Killing
for Elimination	for Conviction

These could possibly be compressed to

for greed, jealousy, fear and hate

although sometimes there may be a mixture as is seen in the case of R. V. Attard (7) (noted later) a revenge killing which was a sequel to the murder of big Audrey by Gopolan for jealousy.

In connection with murderers, Tennyson Jesse discussed what she calls the "Moral imbecile" which she is careful to point out is not necessarily synonymous with the "moral defective." She quotes Tredgold (8) as writing a new definition and says that the "mental *defect*" of the definition was intended to denote, and does denote a defect "entirely different from the ordinary unintelligence of the feeble minded or imbecile," for the moral imbecile is by no means unintelligent in the ordinary sense for he has wisdom which is combined in some cases with abnormal instincts and this is an essential characteristic of the class.

She quotes Mercier (9, 10, 11) as saying in this connection "however astute, clever, dextrous, intelligent, his shifts and dodges and stratagems and tricks may be, he is always a failure in the long run. He may succeed in his immediate object but his devious path soon leads him round into disaster. Usually the diameter lies so plainly before him that we wonder how a man so superficially clever should be beneath the surface such a fool. He takes the most careful measures against detection by one method and lays himself open to certain and speedy detection by another. He has plenty of intelligence but little or no wisdom, he is a clever fool. Such modern characters as Haigh, Christie and Hume certainly show this. Haigh having brilliantly succeeded in liquidating (literally) two complete families without it being even noticed for they had no proper roots, then, of all persons, selected a rich elderly woman who is the regular member of a bridge four in a Kensington Hotel whose absence is bound to be commented upon. As though this was not enough, after dissolving her body in acid he goes no further than an adjoining town to dispose of her property (R v. Haigh) (12). Christie's activities, though apparently for a different kind of gain, also involved repetitive murder of girls who were not likely to be missed or who if their absence was reported to the police, would be considered to have adequate reasons of their own for disappearance. In his case his surrender of the lease of his rooms was found to lead to the discovery of the bodies although it may be said that he had good reason for leaving the house (R. v. Christie (6, 14)). Hume on the other hand appears to have been incapable of learning from experience, or for that matter, appreciation of his good fortune in the Setty case (R. v. Hume (15)). He too appears to

have repeated his method although on two former occasions it nearly led to his undoing without much concrete gain.

Tennyson Jesse quotes as her own examples Palmer (the poisoner), (16) Neil Cream (17) and George Joseph Smith (18) (the Brides in the bath).

Before citing cases to illustrate the various motives which have occurred in practice, there is one other observation for which Miss Jesse must be given full credit. She suggests that just as certain people seem to be predestined to become murderers under certain circumstances, so too others, it may be said because of their stupidity in not appreciating what is going on, are destined to be murdered—these she describes as born *murderees*. There is considerable support for this theory as for example in the case of R. v Whybrow (19, 20) in which the man's wife having forgiven him once for bigamy then finds evidence of yet another liaison, with this she interferes, and yet when she receives an electric shock under such unusual circumstances as being seated on the lavatory, she suspects nothing until she is lucky enough to survive a further shock from touching the soap dish which her husband has deliberately and ingenuously wired up to an electric power plug.

Of all the motives for murder, GAIN is the most common. Gain, however, is not limited to robbery for it may also include gain from inheritance (although this might be called elimination) or to prevent loss of inheritance or for sexual enjoyment of an unwilling party. For gain by robbery, a case of some interest was that of the boy Hyams who murdered an old lady to obtain a few pounds in order to repay money given him by his schoolmates for fountain pens. This he had deviated from its proper use to purchase a "make it yourself" model aeroplane kit. On his own admission when pressed for the pens or the money back he decided to go out and rob someone. This he did, taking with him a length of iron piping and striking down the first suitable victim causing her death. The weapon actually proved to be his door jack as the weapon showed evidence of paint similar to that on his bicycle (2).

A further example of murder for gain occurred in 1959 (R. v Boswork and Jacobs) (22). This case, as in all the others, has an unusual aspect to contemplate or lesson to be learned. A man named Folkard who was a night watchman at a printing works

lived upon the premises. He had lost one eye and made a practice of going around the premises in the dark. On one evening he joined his wife in bed rather later than usual and on turning on the light she found the front of his shirt to be smothered in blood from wounds of his scalp. He was unable to give any explanation for the injuries. The next morning he was in coma and was removed to a hospital for surgical treatment which was unsuccessful. At autopsy he was found to have vertical lacerations on the scalp which at first sight seemed consistent with colliding with the edges of various racks. One wound on the side had been exised at operation and a fractured skull was revealed beneath it. Examination of the works showed evidence of entry by a window. This was indicated by a palm print on a leather cushion, a thumb print on the window, and a foot print on the roof outside. Subsequently, routine examination of the palm prints of suspects detained by the police, identified the owner who admitted that he had been on the premises with another man who he alleged hit the watchman on the head with a printer's hammer. The second suspect proved to be in prison and had confided in his fellow residents that he had attacked Folkard. He was tried and convicted of capital murder but reprieved. This case was of interest as showing how easily injuries due to violence can be mistaken for accident.

Murder for Revenge is represented in this collection of cases by one of more than ordinary interest. A professional tramp and scavenger called Adams who had long white hair and a beard. He lived in a room which he rented and was most punctual in payment of his rent. In the fall of 1957, as he was in arrears with his payments, entry was forced into his room and his body was found lying on the floor, somewhat decomposed and infected with larvae, with his head crushed and lying in a pool of blood. Examination of the front door downstairs showed splashes on the door and on each side. There was also blood smearing of the wall of the staircase. Examination of the skull of Adams showed obvious tangential cuts such as would be caused by a chopper. Later a man living in the next room was arrested and charged with murder. He admitted having seen Adams dead in his room on the day after he was missing. He gave, as explanation as to why he had told no one, that he had a criminal record and was frightened. The case was moreover complicated by the fact that

several people came forward to say that they had seen Adams alive two weeks after he was supposed to be dead. It was, however, possible to establish an approximate time at which death had taken place from identification of the larvae and stages of development. Collier was convicted of capital murder (he was alleged to have taken some of Adams' money) but was reprieved after a petition which contained a full confession including the information that Adams had *walked* upstairs after being attacked with the chopper (23). The reason for this murder was a desire for revenge by Collier because Adams had kicked his cat.

The second example of murder for revenge is "The case of Big Audrey." This lady practiced the oldest profession in Poplar. One, Gopolan, became enamoured with her and she visited him every Friday. He was disillusioned one day to find that he was not the only one upon whom she bestowed her favours. He accordingly set out armed with a knife, located her in a cafe sitting with her "fiancee"—a Maltese gentleman called Attard, slipped inside, pushed her head forwards with one hand and neatly stabbed her in the back with the other puncturing her aorta. He then disappeared down the road hotly pursued by her friends who eventually caught him on a 'bombed site' and felled him to the ground. One somewhat overenthusiastically took the opportunity to hit his head with a piece of concrete lying near by. He was taken to the London Hospital where a craniotomy was performed but in spite of this he died of a secondary pontine haemorrhage. At the subsequent trial (24) they were acquitted of all charges.

Murder for Elimination is represented in this series by two cases of different character. 'The case of the happy sergeant' occurred in Duisburg and involved the exhumation of the dead man upon whom a court of enquiry returned a decision of *suicide*. When, however, eight months later Emmett Dunne married the widow the case was re-opened and from the appearances of photographs taken the day after his death, it appeared that the hanging had been faked as there was congestion both above and below the mark of suspension. Also a most unusual injury was a fracture of the thyroid cartilage in the mid line. Experiments showed that this could be due to an unarmed combat blow on the neck. Later Emmett, a stepbrother of Emmett Dunne, made a statement to the effect that he had helped his stepbrother to hang up Watters

after death. The trial by General Court Martial took place in Dusseldorf and resulted in conviction of Emmett Dunne (25).

The second case was that of the killing of Mrs. Christophilus by her mother-in-law in order that she should remain in England. The background of the case is of importance. Mrs. Christophilus was a Cypriot whose son, a very successful waiter in England, had married a German refugee. The mother who was almost a classical picture of the Matriarch, came to England on the guarantee of her son. It was not very long before she tried to rule the house, a manoeuvre which was naturally resented by the daughter-in-law. As it appeared quite an insoluble problem, the son told his mother to return to Cyprus and that he would withdraw his guarantee for her residence in England. Shortly after this Mrs. Christophilus hailed a passing car outside her front door one night and told him that her daughter-in-law had come to harm. It required no more than a cursory examination of the body which was lying in the back yard behind the house, to decide she was dead and that she had not burnt herself. The body, which appeared to have been soaked in paraffin, was severely charred and examination of the neck showed the mark of a scarf which had been present during the fire and removed afterwards, cutting the skin. At autopsy, an extensive fracture of the skull was seen involving the occipital bone and the base. Reconstruction showed that apparently the lady had first been hit by a heavy weapon (probably the front of the grate). After this the scarf was applied tightly and the body ignited. The investigation of the case was assisted by a neighbour who had seen a fire and looked down into the yard to see Mrs. Christophilus stoking a the fire. "The case of the burning lady" ended in the conviction of Mrs. Christophilus at the Central Criminal Court (26) and her subsequent execution. Information was received that in the past in Cyprus she had been acquitted of a somewhat similar charge.

Of all motives, *Jealousy* is probably the most intense and although often misplanned has led to the most dramatic cases such as R. v Ley and Smith (27, 28) when Ley, a prominent Australian politician and business man, was convicted of the murder of a barman called Mudie whom he believed to be seducing his mistress.

The first case of interest in my series is "the case of the body in the boot" when Ozilins, a Pole, was convicted of murder of an-

other Pole and disposing of his body by dumping it by the roadside. The injuries were of interest consisting of a severe crush injury of the skull and also of the chest due it was alleged by the prosecution, to deliberately running the man over with a car. The evidence of 'dumping' was supplied by a completely dry mackintosh beneath the body when since early evening it had been raining heavily. Although the exact place of the murder was never identified, two important pieces of evidence undoubtedly helped to decide it—firstly, an alibi of a visit to a public house which had clearly not taken place and secondly, the chance that a doctor happened to be passing the spot where the body was dumped and recognised the car (29).

"The case of the flying smuggler" needs little recapitulation as it has been published and more recently revived by the exploits of the central figure in Switzerland. It was chiefly remarkable on account of the method of disposal of the body by cutting it up and throwing the pieces from an aeroplane. Hume finally pleaded guilty to a lesser charge after a retrial (the first trial stopped because of the illness of the Judge) and disagreement of the Jury. On his release from prison, Hume wrote a confession of guilt for the newspapers (15).

Murders from *lust of killing* when the murderer is fully sane must be rare. In "the case of the frightened boxer" Christie appears to have pursued a most consistent path of premeditated murder in order to satisfy his sexual desire. Finally, he proved true to type by leaving his house where he alone could preserve his guilty secret. As was inevitable, another occupant of the house commenced to clean up the room. On stripping the wall paper he discovered a cupboard containing the bodies of three women. Later search revealed another body (Christie's wife) beneath the floor boards of the front room and two skeletons in the garden as evidence of his earlier frolics. The fact that a man called Evans was convicted and executed for the murder of his wife and child in the same house between the first and second periods of Christie's activities, certainly caused more controversy than has been seen for a long time and undoubtedly influenced the modification of the death penalty (6, 14).

Finally, there is the "*murder from conviction.*" Probably the best example of recent years in England was that for which Fantle was convicted.

The two cases in this series might be otherwise classified but can be nevertheless included in this category. In "the case of the empty house" a child who had been missing for 24 hours was found dead in a bomb damaged house. The motive was really elimination for the woman who killed the child is believed to have done so in order to have her husband executed. She got the child into the house and killed it with a hammer. In the process whilst gripping the child, she inflicted grouped linear bruises and abrasions on the child's chest which ultimately was her undoing for examination of her finger nails showed wool fibres similar to those of the child's jumper (30).

The second example is a case of what has been aptly described as a typical "kitchen sink" murder. A spinster who went to stay with her sister-in-law for a short time remained as a permanent lodger at a very low charge. In addition she imposed upon the unfortunate woman to such an extent that one morning unable to bear it any more as she saw her "arrogant sister-in-law" going towards the front door, she seized her scarf from behind and pulled it tight around her neck. The untimely visit of the postman made death quite inevitable, for to stop any cry she placed her hand over the woman's face. This behaviour could well be the outcome of grievous provocation but the next manoeuvre showed considerable presence of mind, for she placed the body in an invalid chair, concealed it in a shed and in the early hours of the next morning dumped it by the roadside to look like a motor accident. Her success was frustrated by the presence of a linear mark of pressure whitening across the back of the leg which was on the wrong surface of the body (31).

All these cases bear out the theories of Tennyson Jesse and in addition have at least one point of original interest to those whose profession it is to practise the detection of crime.

REFERENCES

1. Jesse, F. Tennyson. Murder and its Motives, William Heineman Ltd., London, 1924.
2. Pearson, Edmund Lester: Studies in Murder, The Macmillan Company, New York, 1924.
3. Pearson, Edmund Lester: Murder at Smutty Nose, William Heineman Ltd., London, 1927.
4. Roughead, William: The Riddle of the Ruthvens, W. Green & Son, Edinburgh, 1919.
5. Roughead, William: Famous Crimes, Faber & Faber, London, 1935.

6. Jesse, F. Tennyson (ed.): Notable British Trials—Trials of Christie and Evans, W. M. Hodge, Edinburgh.
7. Regina v. Attard: Central Criminal Court, 1958.
8. Tredgold, A. F.: Mental Deficiency.
9. Mercier, C.: Conduct and its Disorders.
10. Mercier, C.: Crime and Criminals.
11. Mercier, C.: Criminal Responsibility.
12. R. v. Haigh, Lewes Assizes, 1953.
13. Hodge, William: Notable British Trials—Trial of J. G. Haigh, Edinburgh.
14. Camps, Francis E.: Medical and Scientific Investigation in the Christie Case, Medical Publications Ltd., London, 1953.
15. R. v. Hume: Central Criminal Court, 1949.
16. Hodge, Em., Eric R. Watson (ed.): Notable Trial Series—Trial of William Palmer, Edinburgh, 1856.
17. Shore, W. Teignmouth (ed.): Trial of Neil Cream, 1892.
18. Watson, E. R. (ed.): Trial of George Joseph Smith, 1915.
19. R. v. Whybrow: Essex Assizes, 1952.
20. Camps, F. E. and W. P. B. Purchase: Practical Forensic Medicine, Medical Publications Ltd., 224, 1956.
21. R. v. Hyams: Central Criminal Court, 1957.
22. R. v. Bosworth & Jacobs: Central Criminal Court, 1958.
23. R. v. Collier: Central Criminal Court, 1958.
24. R. v. Attard: Central Criminal Court, 1958.
25. R. Emmett Dunne: General Court Martial, Dusseldorf, 1955.
26. R. v. Christophilus: Central Criminal Court, 1954.
27. R. v. Ley & Smith: Central Criminal Court, 1947.
28. Roberts, C. E. Bechhoffer (ed.): The Trial of Ley Smith, Jarrolds, London.
29. R. v. Ozilins: Central Criminal Court, 1957.
30. R. v. Tierney: Central Criminal Court, 1948.
31. R. v. Chubb: Central Criminal Court, 1958.
32. Bedford, Sybil: The trial of Dr. Adams, Simon and Schuster, 1959.

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AMERICAN SCIENCE AND OUR NATIONAL PROBLEMS[a]

CHAUNCEY D. LEAKE[b]

The major national problems confronting the United States are due to the nuclear age, over-population, the consequences of extensive industrialization, and the prolongation of life expectancy, all of which are brought about by the tremendous expansion of our scientific effort.

Along with these specific problems, which are becoming important for peoples all over the earth, is the more difficult problem of preserving the dignity of individual human beings in the face of increasing authoritarianism and of increased organization bureaucracy.

Our major problems, which are capable of scientific approach, remain the necessity for more verifiable information, and thus better understanding, about ourselves on the one hand; and our environment on earth and in the universe on the other hand.

Top priority should be given to more support, sympathy, appreciation, and better social recognition for *basic scientific research*. Basic science is concerned with getting verifiable information about ourselves and our environment.

It is essential then to analyze and interpret this scientific knowledge freely for the benefit of the peoples of the world. This is a major scientific problem confronting us, dealing with the essentials of information theory, and of effective communication. We have to translate what science is about and what it is doing to our people in the language that they can understand.

If our available scientific knowledge about ourselves and our environment is freely available to our people, we can then perhaps wisely and with good will reach satisfying policy agreements on our major social problems:

(1) Over-population;
(2) Control of nuclear energy;
(3) Pollution of airs and waters;
(4) Conservation of natural resources and the preservation of the inherent beauty of our good earth;
(5) Removing the continuing threat of authoritarianism;

(6) Preserving the dignity of individual human beings;
(7) Promoting individual responsibility for self-sufficient old age;
(8) Promoting optimum physical and mental health;
(9) Preserving our comforts, conveniences, and cleanliness, with equal opportunities for all in decent living, and
(10) Understanding and controlling our individual and group prejudices, fears, anxieties and ambitions, so that we all can enjoy the good life possible for us.

Rockets to the moon are spectacular, but what for? Miracle drugs are wonderful for curing disease, but then what? Huge over-powered automobiles are thrilling to ride, but what then?

Maybe it would be wise for us to consider from time to time what science is really about, in contrast to the distortions which may occur in the commercialized applications of scientific information. The silly advertising of a lot of different brands of aspirin may be indicative of our sense of values. Maybe we need to blame our sciences by the humanities: maybe we would be wise to consider our purposes, our motives, and then the wisdom of our judgments.

We need desperately to learn a lot more about ourselves. We can then approach the knowledge we have of our environment with better possibility of utilizing it effectively for our own welfare.

How do our brains work? What determines our individual and group behavior? These are basic scientific problems, and they deserve the most intensive support and study.

How can we learn to get along with each other, and as a nation with other nations? Are we headed for Aldous Huxley's *Brave New World* or for Orwell's nightmare, or is there some hope for individual dignity and decency, with freedom and responsibility for each of us?

This is a time of intellectual crisis. Our value judgments are in chaos. This is inevitable when great new scientific principles develop and are being assimilated by people generally. The development of quantum theory and of nuclear physics give us the power of nuclear energy: shall we use this for arrogant threats and for death or destruction or for the peaceful benefit of all people?

The application of quantum theory to biology may be equally

as disturbing: what are the prospects of producing genetic changes in living things in specific directions and toward specific ends? Can we actually control human heredity? Does our increasing knowledge of the makeup of genes, viruses, and the organization of living cells, permit us to approach still further an appreciation of life in terms of physics and chemistry? Can we utilize our increasing knowledge of viruses to the effective control of immunity, and thus to freedom from disease, and even from cancer? But what is going to happen if we all live too long?

This then is a time of intellectual crisis. We have so much new science to assimilate that we are bound to have some intellectual indigestion. A major problem exists in translating the developments of current science so that people generally may understand them, and realize their consequences. We have increasingly, each of us, the obligation to learn about the vastly complex factors which in our brain-stems determine our emotions, and which in the coverings of our brains, determine our thinking and reasoning.

Science then is a vast cooperative undertaking between technical experts and people everywhere. To be successful in benefiting us all, it becomes increasingly necessary for us to agree on the purposes to which we apply our science. Maybe as we learn more about ourselves we can come more easily to mutually satisfying agreements between individuals or between groups of people.

[a] Presented at a luncheon sponsored by Hartz Mountain Products, Corporation, New York, marking the publication of "Perspectives in Virology", (Morris Pollard, Editor) John Wiley and Sons, New York. Accepted for publication April 9, 1959.

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PROCEEDINGS, TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

Abstracts of Some of the Papers Read at the Spring Meeting

April 17–18, 1959

Texas State Department of Health, Austin

An Evaluation of Several Media for the Early Detection of *Pseudomonas Aeruginosa* Encountered in Clinical Practice.

Irving Davis and Walter Sellers, Department of Microbiology-Cellular Biology, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas, and
Hyman Orbach and George Weddington, USAF Hospital, Clinical Laboratory, Lackland Air Force Base, Texas.

The characteristic pigment produced by *Ps. aeruginosa* may be considered pathognomonic when viewed in plate cultures of clinical specimens. The effectiveness of this rapid and reliable diagnostic criterion is diminished because of the failure of many strains of this species to produce pigment on the isolation medium. Comparisons of the qualitative and quantitative pigmentation produced by the same 102 strains of *Ps. aeruginosa* on several media were independently completed in the two laboratories. The deficiencies possessed by the usual laboratory media as represented by nutrient agar were apparent in both laboratories. On this medium greater than 50 percent of the strains failed to show pigmentation and the laboratories qualitatively disagreed on approximately 50 percent of the specimens. The ability of Sabouraud maltose agar to increase the changes for qualitatively detecting pigmentation, as well as increasing the degree of pigmentation of this species, were noted by both laboratories. Ninety-two percent of the strains showed pigment on this medium; only three percent disagreement was observed between the laboratories. It is suggested that careful consideration be given to the routine use of Sabouraud maltose agar in diagnostic bacteriologic procedures in selected clinical specialties.

Growth Enhancement of *Lactobacillus Casei* by *Candida Albicans* and Saliva.

Thomas E. Wilson and Paul W. Goaz, Dental and Microbiological Research Section, Oklahoma Medical Foundation, Oklahoma City, Oklahoma.

When vitamin deficient medium is conditioned by *Candida albicans* for 20 hours in a diphasic culture system, the subsequent growth of the lactobacillus is proportional to the amount of vitamins released. Addition of saliva to the vitamin deficient medium results in growth that is generally proportional to

the amount added. This growth cannot be entirely attributed to the vitamin content, since an increase in maximum growth, roughly proportional to saliva concentration, was also observed in the complete medium. Conditioning complete medium with candida reduced the initial stationary phase 40 per cent and increased the growth rate 30 per cent. The addition of 10 per cent and 25 per cent autoclaved whole saliva to the medium, shortened the initial stationary phase 20 per cent and 30 per cent respectively, and both concentrations increased the growth rate 35 per cent. Conditioning the medium for periods up to three days had a negligible effect upon the maximum growth of the lactobacillus; however, longer periods caused some increase. When saliva supplemented medium was conditioned by candida, the initial stationary phase was further reduced, but the effect was less than additive. No complementary effect upon growth rate, or maximum growth was demonstrated.

Variation in Sensitivity of *Serratia Marcescens* Strains to Ultraviolet and X-ray Irradiation.

Robert J. Ball and Robert P. Williams, Department of Microbiology, Baylor University College of Medicine, Houston, Texas.

Two strains of *Serratia marcescens* were exposed to the same dosage of both X-ray and Ultraviolet irradiation. The number of survivors to X-ray was identical for both strains, but one strain showed 10^3 more survivors to ultraviolet than the other. The more resistant strain also demonstrated a much greater catalase activity. When exogenous catalase was added to both strains during irradiation, a 10 per cent increase in survivors was noted, but one strain was still 10^3 times more resistant to ultraviolet.. The reason for the difference in susceptibility to ultraviolet light is unknown at this time. The results suggest that the factors determining susceptibility or resistance to irradiation might be different for X-ray than ultraviolet light.

A Quantitation of Certain Factors Which Influence the Survival of *Escherichia coli* in Aqueous Solutions of Ozone.

Irving Davis, Department of Microbiology-Cellular Biology, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.

The effect of varying ozone and cell concentration, and ozone-cell contact times on the survival of *E. coli* was studied. Ozone was produced by a Welsbach water-cooled corona discharge ozonator using dry, clean oxygen. Cells were exposed to aliquots of stock solutions of ozone. Survival was determined by recognized plating procedures. Maximum lethal effects of ozone over a wide concentration range (.1 to 1.5 mcg. per ml.) were expressed in one minute or less on the bacterial population (approx. 2×10^9 cells per ml.). Lethality in this range of ozone was from 63 to 99.5 per cent. The number of cells surviving exposure is a function of approximately the cube of the initial cell concentration. This holds true over a wide range of ozone values (.1 to

1.74 mcg. per ml.). A plot of data for per cent survival as a function of ozone concentrations showed extreme variations. A probit analysis was completed based on the assumption that tolerance to log concentration of ozone is normally distributed in the population. Per cent kill increased sharply over a narrow range of ozone concentrations (0 to .4 mcg. per ml.) and then leveled off. The ozone tolerance of strains B and B/r of *E. coli* appear to be similar, if not identical.

Endogenous and Polyphenolase Rates of *Streptomyces Griseus* Cells as Affected by Streptomycin. V. Feisal and G. E. Peterson, Department of Biology, University of Houston, Houston, Texas.

Thrice washed (0.05 M phosphate; pH 7.0) cells of *Streptomyces griseus* ATCC 10137 from 2, 3, 4, and 5-day old pilot scale streptomycin fermentations were tested manmometrically for the reaction of their endogenous respiration to streptomycin (640 mg free base/ml). Endogenous rates per unit dry weight were high in all cells. However, cells from 48 and 110-hour fermentations used more oxygen than did the material from cultures which were 72 and 92 hours of age.

Streptomycin inhibited oxygen uptake by 48-hour cells but had little effect on 72 and 92 hour old growth. In contrast, the oxygen uptake by cells from 110 hour fermentations increased by a significant quantity ($p=0.04$) in the presence of streptomycin. However, cells of the latter type failed to: 1) grow in a synthetic medium containing streptomycin as a sole source of carbon; 2) remove any streptomycin from a 0.05 M phosphate solution of the drug over a contact period of 96 hours.

The endogenous respiration of 110-hour cells was reduced to nearly zero by washing and reacting the cells at pH 4.3 rather than pH 7.0. No such effect was noted in cells processed at pH values of 5.0 and 6.0. The endogenous rate of pH 4.3 cells was not stimulated by the addition of streptomycin. Conversely, the endogenous rate of the pH 7.0 cells was unaffected by an oxidase substrate consisting of hydroquinone and a catalytic amount of catechol; the pH 4.3 cells readily oxidized such a mixture. A combination of streptomycin and the polyphenolase substrate caused a marked depression of endogous oxygen uptake by pH 7.0 cells but had no effect on the polyphenolase activity of the pH 4.3 processed growth.

When cells from a 5-day fermentation were washed at pH 4.3 and reacted at pH 7.0 the endogenous rate remained negligible. However, the polyphenolase activity of cells so treated was markedly reduced by the addition of streptomycin.

Further Studies on the Evaluation of Media for Root Canal Culture. William A. Nolte, Department of Microbiology, The University of Texas Dental Branch, Houston, Texas.

Brewer's thioglycollate, brain heart infusion, trypticase soya, and micro inoculum broths, plus 0.2% agar, were evaluated to determine the effect of

added starch, yeast extract and ascitic fluid on growth of a strain of fusobacterium, streptococcus, lactobacillus, actinomycete and candida.

Added 1% starch to these media was stimulatory for growth for the fusobacterium, lactobacillus and actinomycete. One exception was noted, added starch to trypticase soya was slightly inhibitory for growth of the actinomycete. No growth stimulation was observed for the streptococcus or candida by the addition of starch.

The addition of 2% yeast extract, to brain heart infusion plus 1% starch, stimulated growth of the actinomycete, fusobacterium and lactobacillus only. The addition of yeast extract to trypticase soya plus 1% starch was stimulatory for the lactobacillus and actinomycete only; whereas, the addition of yeast extract to Brewer's plus 1% starch, was stimulatory for the actinomycete only.

No growth stimulatory effect was observed for any of the organisms by the addition of yeast to micro inoculum plus 1% starch.

Micro inoculum plus 0.2% agar and 1% starch was as sensitive, as stimulatory as, or more stimulatory than, any of the other media when tested alone or plus starch, yeast extract, or ascitic fluid, for growth of the organisms used in this study.

The Sensitivity of Mixed Populations of Bacteria to Inhibitors.

R. H. Bauerle and E. O. Bennett, Department of Biology, University of Houston, Houston, Texas.

A study was made concerning the effects of mixed populations of *Pseudomonas aeruginosa* and *Desulfovibrio desulfuricans* on the sensitivity of the individual species to three groups of bacterial inhibitors. It was found that the presence of *P. aeruginosa* caused in some cases a 100% increase in resistance of *D. desulfuricans* to substituted phenols, a consistent increase in resistance of *D. desulfuricans* to nitroparaffins, often as high as five fold, and no marked effect on their sensitivity to mercurials.

The presence of the sulfate-reducing bacteria caused an increase in resistance of the pseudomonads to some of the nitroparaffins, ranging as high as 33%, a consistent increase in resistance of the pseudomonads to mercurials, in one case as high as 16 fold, and a consistent increase in sensitivity of the pseudomonads to phenols, often as high as 30%.

The use of dead *P. aeruginosa* cells in the mixtures had no effect on the sensitivity of sulfate-reducing bacteria to phenols and nitroparaffins. The presence of dead sulfate-reducing bacteria produced an increase in resistance of the pseudomonads to mercurials and nitroparaffins but unlike the live cells, did not increase the sensitivity of the pseudomonads to phenols.

Limits of Typability of Coagulase Positive Staphylococci.

C. E. Dowell and E. D. Rosenblum, Department of Microbiology, University of Texas Southwestern Medical School, Dallas, Texas.

Fifteen new phages isolated from lysogenic staphylococcal strains were able to type 75 (54%) of 139 of untypable coagulase positive staphylococcal strains.

To see if more new phages could be isolated to type the remaining 64 untypable strains, supernatants were obtained from 74 strains of known phage type and spotted on the 64 untypable strains. The 64 untypable strains were also cross spotted. Thirty-one, or an additional 22%, showed sensitivity to temperate phages.

We felt it might be possible to assign strains to a phage group on the basis of the host range of their carried phage or lysogeny. Therefore we spotted supernatants from the 64 untypable strains on 74 strains of known type. Forty-six or an additional 33% showed lysogenic activity on the typable strains.

Out of a total of 139 untypable strains, only 10 or 7% were not typable by any method.

Biological Activities of Certain Phage Typable Staphylococci.

Willson J. Fahlberg and J. Marston, Department of Microbiology, Baylor University, College of Medicine, Houston, Texas.

A biological comparison of ninety freshly isolated pathogenic strains of *Staphylococcus pyogenes* was made. On the basis of phage typing, four groups were compared: phage type 44A, phage type 44A/80/81, a heterogenous phage-typable group containing neither 44A or 44A/80/81, and a non-typable group. The following biological measurements were made: Free-coagulase, Bound coagulase, Pigment, Mannitol, Fermentation, Delta lysin, Alpha lysin, Beta lysin, Fibrinolysin, Phosphatase and Urease.

Significant increases were observed in the production of: Beta lysin with the non-typable group, Fibrinolysin with the non-typable group, and urease with 44A and non-typable groups. Delta lysin activity was markedly decreased in group 44A/80/81. Other biological activities were comparable and generally in accord with the literature.

Lysogenic Conversion of Strain 80/81 and Related Staphylococcal Phage Types.

J. L. W. Jackson and E. D. Rosenblum, Department of Microbiology, The University of Texas Southwestern Medical School, Dallas, Texas.

During an epidemic caused by strains of phage type 80/81, a related phage pattern (52/52A/80/81) was observed and strains with this altered typing pattern seemed to be increasing. Strains isolated during the same outbreak were screened for temperate phages active against strains of phage type 80/81 and 52/52A/80/81. Two temperate phages were isolated, phage 53 being active against both phage types whereas phage 60 acted only upon strain 52/52A/80/81.

Lysogenization of the typical 80/81 strains with phage 53 made them sensitive to the typing phages 52 and 52A, and to temperate phage 60. This seems to indicate that 80/81 strains are carrying a phage 60-like prophage which is replaced by lysogenization with phage 53, resulting in the increased

phage pattern 52/52A/80/81. The presence of this prophage is still speculative and further screening with additional indicator strains to demonstrate its presence is planned.

The Pathogenesis of Rabies in Gravid and Non-Gravid Insectivorous Bats (*Tadarida mexicana*).

Ruth A. Sims, Rae Allen and S. Edward Sulkin, Department of Microbiology, The University of Texas Southwestern Medical School, Dallas.

A previous report from this laboratory presented evidence that the brown adipose tissue of the Mexican free-tailed bat may be a storage depot for rabies virus, harboring the virus during periods of latency. The present study is concerned with the influence of the reproductive cycle on the pathogenesis of rabies in these animals.

Comparison of results of experiments with bats inoculated intramuscularly with rabies virus in early, middle and late pregnancy with results of an earlier experiment using non-gravid bats indicated that experimental infection of these animals with rabies virus is not significantly influenced by the gravid state. Possible transplacental transmission of rabies virus was oberved and in two instances virus was isolated from mammary tissue. The low infection rates (20–30%) observed following intramuscular inoculation of massive doses of this agent suggest the relative insusceptibility of *Tadarida mexicana* to experimental rabies infection.

Findings on Another Outbreak of Ornithosis.

Betty McGrew, Kinch Knolle, and Carl D. Heather.

The flock of turkeys in question originated in San Saba County and consisted of 2,300 poults. From these poults, 1,500 market birds were raised. They became infected about December 15 to the 21, 1957. About 355 of these birds died between December 21, 1958 and January 28, 1959. On two different occasions these birds were diagnosed as having typhoid by poultry service men. On February 4, 1958, Texas A & M College reported the isolation of ornithosis virus from members of this flock. Five weeks after infection, the birds were processed. The processing of these birds resulted in 11 cases of laboratory confirmed human ornithosis among the poultry plant employees.

THE PHARMACOLOGICAL ACTION OF AUTONOMIC DRUGS ON THE EYE OF FOWLS*

MELVIN E. CLOUSE AND R. H. RIGDON[a]

The effect of parasympatholytic and sympathomimetic drugs on the eye of man is well known. Mydriasis results from either the contraction of the dilator muscle or the relaxation of the sphincter muscle in the iris (3). These changes result from either stimulation of the sympathetic or inhibition of the parasympathetic nervous system. The sphincter and dilator muscles in mammals are composed of smooth muscles (1); however, striated muscle is present in the iris of birds (6).

Few experimental observations have been made of the pharmacological action on the pupil of birds of the drugs frequently used in man. In 1893 Meyer (5) observed that a 5 to 10 per cent concentration of atropine sulfate, when put into the conjunctival sac of the chicken, caused a cessation of all movements of the iris; however, a lesser concentration had no effect. Sollmann (7) also stated that atropine sulfate has no effect upon the striated muscle in the iris of the fowl but curare produces mydriasis. Meyer (5) observed that a 1 per cent solution of curare caused dilation of the pupil in the dove.

During a study of cataracts in the chicken and turkey, it was found that the drugs usually used to produce mydriasis had no effect on the pupil. Experiments were then made to observe the effect of several parasympatholytic and sympathomimetic drugs on the normal eye of fowls.

Methods and Material

The birds used in this experiment were young adults. There were five white Leghorn chickens, five white Pekin ducks and five turkeys. The following concentrations of drugs were used: d-tubocurarine chloride (Tubocurarine) (3 mg/ml); chondodendron tomentosum extract, purified (Intocostrin) (20 units per ml); epinephrine (1.0%); tetracaine hydrochloride (Pontocaine) (0.5%); atropine sulfate (1.0%); and phenylephrine hydrochloride (Neosynephrine) (10.0%). Each of these drugs was placed on the cornea of each of the fifteen birds every three minutes for one hour. Physostigmine salicylate (0.25%) was placed on the cornea of each chicken, duck and turkey at intervals of 15 minutes for one hour. All observations were duplicated.

Each of the above drugs in the same concentrations except for epinephrine

(0.1%) and carbamylcholine chloride (Carbachol) (0.01 and 0.1%) was injected into the anterior chamber of the eye of the chickens and ducks. Tubocurarine, Intocostrin and Pontocaine were injected into the anterior chamber of the rabbit's eye. Approximately 0.05 ml of fluid were withdrawn from the anterior chamber with a tuberculin syringe and a 30 gauge needle. With the needle still in place, 0.05 ml of medication were injected into the chamber. The reaction of the pupil was observed with direct light either with or without a dissecting microscope.

The iris was removed from normal chickens for histological demonstration of the muscle and fixed in a 4.0 per cent solution of formaldehyde. Paraffin sections were prepared and stained routinely with hematoxylin and eosin. Select sections were stained by Mallory's technique.

Observations

The normal iris in the chicken is shown in Figure 1. There is a wide variation in the proportion of fibrous tissue and striated muscle (Fig. 2) in different areas of the same iris. The muscle fibers in the iris are primarily circular (Fig. 1A). Only a few muscle fibers run in the opposite plane (Fig. 1B).

The effect of the drugs used in this study is summarized in Table 1. Tubocurarine and Intocostrin in the concentrations used when placed on the cornea of the chicken, duck and turkey produced no visible effects on the pupil. However, when they were injected into the anterior chamber, mydriasis immediately occurred and the pupil did not react to light. When these same two drugs were injected into the anterior chamber of the rabbit's eye, the pupil became fixed with neither dilatation or constriction and the pupil did not react to light.

The pupil in the chicken, duck and turkey did not react to light when a 1.0 per cent solution of epinephrine was put on the cornea. However, mydriasis did occur in the chicken and duck immediately after a 0.1 per cent solution of epinephrine was put into the anterior chamber and dilatation persisted for a period of 20 minutes. The pupils did not react to light during this time. Mydriasis occurred in the rabbit within 20 minutes after a 1.0 per cent solution of epinephrine was put on the cornea.

EXPLANATION OF FIGURES

FIG. 1. Iris in the chicken to show the circular (A) and longitudinal muscle (B). H&E stain, X 150.

FIG. 2. The muscle in the iris of the chicken is striated. H&E stain, X 270.

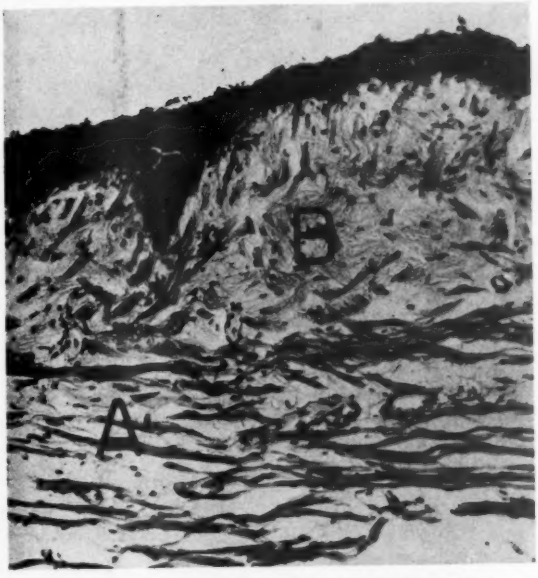
B
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TABLE 1

Pupillary Response to Local Corneal Application of Mydriatic and Miotic Drugs

Drugs	Concentration	PUPILLARY REACTION															
		Chicken				Turkey				Duck				Rabbit			
		Number eyes used	Mydriasis	Miosis	No response	Number eyes used	Mydriasis	Miosis	No response	Number eyes used	Mydriasis	Miosis	No response	Number eyes used	Mydriasis	Miosis	No response
Tubocurarine	0.3%	10	0	0	20	10	0	0	10	10	0	0	20	8	0	0	8
Intocostrin	20 units/ml	10	0	0	20	10	0	0	10	10	0	0	20	8	0	0	8
Epinephrine	1.0%	10	0	0	20	10	0	0	10	10	0	0	20	8	8	0	0
Pontocaine	0.5%	10	20	0	0	10	10	0	0	10	20	0	0	8	8	0	0
Atropine Sulfate	1.0%	10	0	0	20	10	0	0	10	10	0	0	20	8	8	0	0
Neosynephrine	10.0%	10	0	0	20	10	0	0	10	10	0	0	20	8	8	0	0
Physostigmine salicylate	0.25%	10	0	0	20	10	0	0	10	10	0	0	20	8	0	8	0

Mydriasis occurred within 15 minutes after Pontocaine was placed on the cornea of the chicken eye and persisted for 45 minutes; it occurred within 25 minutes in the duck and persisted for 60 minutes. Dilatation occurred in 25 minutes in the turkey and persisted for 30 minutes. When Pontocaine was injected into the anterior chamber of the eye of the chicken and duck, dilatation of the pupil occurred within 10 minutes and persisted for two hours. Mydriasis occurred in the rabbit eye within 15 minutes and persisted for two hours when Pontocaine was placed on the cornea. When Pontocaine was injected into the anterior chamber of the rabbit eye, mydriasis occurred within two minutes.

No visible effects were observed in the pupil of the chicken, duck and turkey following the local application of atropine sulfate to the eye; however, when atropine sulfate was injected into the anterior chamber, the pupil dilated only slightly and was insensitive to light. Mydriasis occurred within one hour when atropine sulfate was put on the cornea of the rabbit; it persisted for 24 to 48 hours. During this time the pupil did not react to light.

Neosynephrine, when placed on the cornea of the chicken, duck and turkey, produced no effect on the pupil; but mydriasis occurred immediately when it was injected into the anterior chamber. Dilatation of the rabbit's pupil occurred within 20 minutes following the local application of Neosynephrine to the cornea.

Physostigmine salicylate when put directly on the cornea produced no effect upon the pupil of the birds used in these experiments. Miosis did occur within 15 minutes when it was placed on the cornea of the rabbit and the pupil remained contracted for ninety minutes.

A 0.1 per cent concentration of Carbachol produced mydriasis within 40 minutes when placed on the cornea of the chicken and duck. Miosis occurred immediately when 0.1 per cent Carbachol was injected into the anterior chamber. However, within two minutes complete mydriasis was present and persisted from 20 to 30 minutes. A similar reaction of the pupil occurred following the injection of 0.01 per cent Carbachol. In the rabbit, injection of a 0.01 per cent solution into the anterior chamber produced prompt miosis and the pupil remained insensitive to light.

TABLE 2

Pupillary Response to Injection of Mydriatic and Miotic Drugs into the Anterior Chamber

Drugs	Concentration	PUPILLARY REACTION											
		Duck				Chicken				Rabbit			
		Number eyes used	Mydriasis	Miosis	No reaction	Number eyes used	Mydriasis	Miosis	No reaction	Number eyes used	Mydriasis	Miosis	No reaction
Intocostrin	20 units/ml	2	2	0	0	1	1	0	0	4	0	0	1(a)
Epinephrine	0.1%	1	1	0	0	1	1	0	0	.	.	.	.
Atropine Sulfate	1.0%	2	2	0	(b)	2	2	0	(b)	.	.	.	.
Neosynephrine	10.0%	1	1	0	0	1	1	0	0	.	.	.	.
Carbachol	0.01%	1	0	2	(c)	1	0	1	(c)	.	.	.	.
Carbachol	0.1%	1	0	1	(c)	1	0	1	(c)	1	0	1	0
Pontocaine	0.5%	1	1	0	0	1	1	0	(c)	4	0	0	(d)
Procaine	1.0%	.	.	.	.	2	2	0	0	2	0	0	2
Tetraethylammonium Chloride		.	.	.	.	2	2	0	0	.	.	.	.

(a) Pupil became fixed and did not react to light.
(b) Pupil dilated slightly and remained insensitive to light.
(c) Miotic within 2 minutes then mydriatic.
(d) Minimal mydriasis with reaction to light.

Discussion

Mydriasis occurs immediately following the local injection of both epinephrine and Neosynephrine into the anterior chamber of the chicken eye but no pupillary change was observed when these drugs were put on the cornea. This variation in the reaction of the pupil may be due to a failure of absorption or to the mechanical removal of the drug by increased lacrimation.

Mydriasis occurs when Intocostrin is injected into the anterior chamber of the bird's eye. Meyer (5) observed this same reaction in the dove when a 1.0 per cent solution of curare was put on the cornea. A 1.0 per cent solution of Intocostrin when put on the cornea of the birds produces mydriasis only after prolonged application. Only a slight degree of mydriasis occurs in the rabbit's eye following injection of Intocostrin into the anterior chamber. Intocostrin produces a paralyzing effect upon striated muscle in man through an interference with the neuromuscular synaptic transmission (7). Since the iris in the bird has striated muscle, it would appear that relaxation of this muscle occurs as a result of some interference with acetylcholine at the effector cells.

The pupil in the fowl dilates only slightly and remains insensitive to light following the local injection of atropine sulfate into the anterior chamber. Meyer (5) in 1893 observed this same reaction when he put a 5 to 10 per cent solution of atropine sulfate in the conjunctival sac. In his experiments the iris remained fixed and all movements ceased. No reaction was observed when a lesser concentration was used. Atropine sulfate produces mydriasis in the human eye by paralyzing the parasympathetic nerve endings (7). Atropine sulfate also paralyzes the mammalian heart muscle and may either slow or quicken the rate of contraction of the frog's heart (7). It would seem that the striated muscle in the iris of the chicken is paralyzed by atropine sulfate, a mechanism suggested by Meyer (5) in 1893. Such an effect would be expected in birds in view of the presence of a striated muscle in the iris.

Pontocaine when injected into the anterior chamber of the chicken eye produces a maximum amount of mydriasis, while a minimal degree of mydriasis occurs in the rabbit's eye. The neuromuscular synaptic transmission is weakened by Pontocaine, according to Sollmann (7). However, Jaco and Wood (4) in 1944 attributed the action of Pontocaine to diminution of acetylcholine

at the neuromuscular junction. Whether the slight dilation, which occurs in the pupil of the rabbit following the injection of Pontocaine, is due to a decrease in the tonus of the smooth muscle of the sphincter pupillae or to an inhibition of acetylcholine at the effector cells cannot be determined from these experiments. Sollmann (7) states that a low concentration of procaine stimulates, and a high concentration decreases, the motility of the smooth muscle in the ileum of the rabbit. It also relieves the spasms resulting from the action of acetylcholine and nicotine. A slight dilatation of the pupil in the rabbit may result from the high concentration of Pontocaine injected into the anterior chamber by decreasing the motility of the smooth muscle in the iris.

Carbachol produces mydriasis in the fowl and miosis in the rabbit. The nicotinic action of Carbachol, according to Goodman and Gilman (2), is more pronounced than that of acetylcholine. The nicotinic effect is stimulation of striated muscle. Dilatation of the pupil in the chicken, following the local application of Carbachol, apparently is due to paralysis of the muscle in the iris. Goodman and Gilman (2) state that paralysis may occur in the presence of high concentration of Carbachol by complete and continuous depolarization of the post-synaptic membrane. The muscarinic action of Carbachol on the mammalian pupil is miosis. It is small, however, compared with the other action of choline derivatives on the pupil. Miosis was observed to occur immediately in the eye of the rabbit following the local injection of Carbachol into the anterior chamber. Such would be expected in the presence of smooth muscle in the pupil. No nicotinic effects of Carbachol were observed in the pupil of the rabbit. Methacholine chloride (Mecholyl), whose action is almost purely muscarinic, produces no visible effect on the chicken iris but miosis occurs promptly when it is injected into the anterior chamber of the rabbit eye.

From this study of the action of different drugs on the iris of the fowl, it would appear that the innervation of the iris of birds is similar to that in man. The different reactions of the pupil of the fowl and man may be explained by the presence of smooth muscle in the iris of man and striated muscle in the iris of the bird. Walls (8), in the study of the development of the iris in vertebrates, has pointed out that the higher fish, snakes and birds

have striated muscle within the iris and that smooth muscle first appears in the iris of marsupial.

Summary

Mydriasis in the fowl is produced by drugs such as epinephine, Neosynephrine, Intocostrin, Tubocurarine, Pontocaine and Carbachol. Apparently the action occurs either through an interference with acetylcholine at the myoneural junction or by direct effect on the muscle.

Essentially no reaction was observed with atropine sulfate on the pupil of the fowl. Apparently, this may be attributed to the presence of striated muscle in the iris.

REFERENCES AND NOTES

1. Arey, Leslie Brainerd: Special Sense-organs, Sec. IX, Morris' Human Anatomy, 10th Ed., p. 956, edited by J. Parsons Schaeffer, The Blakiston Company, Phila., 1942.
2. Goodman, Louis and Alfred Gilman: The Pharmacological Basis of Therapeutics. 2nd Ed., pp. 426, 430, The Macmillan Co., New York, 1955.
3. Houssay, A. Bernardo: Human Physiology, 2nd Ed., p. 956, McGraw-Hill Book Co., Inc., New York, 1955.
4. Jaco, N. T. and D. R. Wood: *J. Pharm. and Exper. Therap.* 82: 63–73, 1944.
5. Meyer, Hans: *Arch. f. Exper. Path. und Pharm.* 32: 101–123, 1893.
6. Smythe, R. H.: *Veterinary Opthalmology*. p. 59, Bailliere Tindall & Cox, London, 1956.
7. Sollmann, Torald: A Manual of Pharmacology, 8th Ed., pp. 382, 446, 329, 332, W. B. Saunders Co., Phila., 1957.
8. Walls, Gordon Lynn: The Vertebrate Eye and its Adaptive Radiation. Cranbrook Institute of Science, Bull. #19, Aug. 1942, The Cranbrook Press, Bloomfield Hills, Mich., 1942.

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ELECTRON MICROSCOPIC OBSERVATIONS OF THE GAMETOCYTES OF A MALARIAL PARASITE (*PLASMODIUM CATHEMERIUM*)[1]

DONALD DUNCAN, JAMES STREET[a] S. R. JULIAN[b] AND DON O. MICKS

Introduction

Fulton and Flewitt (3) published the first study of malaria parasites prepared by buffered osmic acid fixation and ultrathin sectioning. Their work on *P. berghei* and *P. knowlesi* disposed of a long standing controversy on the exact location of malaria parasites with respect to the red cell. According to their observations the parasites are intracellular and not attached to the erythrocytes as claimed by many using the light microscope. In addition Fulton and Flewitt demonstrated the presence of mitochondria and found suggestions of endoplasmic reticulum in the parasites.

The next study of this kind was published by Rudzinska and Trager (7). Their most important observation, made on *P. lophurae*, was the demonstration of a feeding process they called intracellular phagotrophy. By this they refer to the capacity of the parasite to engulf discrete masses of red cell cytoplasm with a subsequent digestion which leaves deposits of malarial pigment (hemosiderin) as the final visible result. In addition Rudzinska and Trager noticed distinct limiting membranes between host and parasite and published much clearer figures of mitochondria, nuclei and endoplasmic reticulum than those obtained in the initial study by Fulton and Flewitt.

In neither of these publications is there a distinction made between gametocytes and the asexual forms, there is no mention of a Golgi apparatus, and no clear statement on the presence of nucleoi. The present study indicates that gametocytes are easily identified in sections studied in the electron microscope, that all forms of the parasite contain nucleoli and that a distinct Golgi apparatus is present in some gametocytes.

Materials and Methods

Parasitemia was built up in canaries by serial passage of infected blood. When a high count of infected cells, 30% or higher, was obtained, blood was

drawn from the infected bird and injected directly into 2% osmium tetroxide solution. Subsequent procedures were much as advocated by Goodman, Reilly and Moore (4). After 20–30 minutes, the fixative was replaced with 65% ethyl alcohol, then 95% alcohol, followed by three changes of absolute alcohol. From alcohol the blood was carried through a half and half mixture of alcohol and plastic monomer, three changes of monomer and finally to catalyzed plastic. Changes were made about every 15 minutes and the cells were lightly centrifuged before each change was made. The plastic was n-butyl methacrylate containing 15% methyl methacrylate. The catalyst was Lupeco CDB. Polymerization was effected by placing the blood and plastic in No. 00 gelatin capsules overnight in an oven at 45°C. Sections were cut with a Porter-Blum microtome. They were collected on uncoated copper grids and subsequently filmed with carbon in a high vacuum evaporator. The material was studied and photographed in an RCA EMU-3 microscope.

Observations

The limiting membrane of the gametocyte is its most striking feature, see Figs 1, 2, 3. In contrast to the faint and often indiscernible double fine lines separating the trophozoites and schizonts from the host cell cytoplasm, the gametocyte is limited by three dense lines separated from each other by two less dense intervals. So far as the authors are aware this is a unique type of cell boundary. In many cells this membrane is partially interrupted by small vesicles which suggest that it is far from a rigid or static structure. As shown in Fig. 1 it is sometimes infolded and surrounds a mass of erythrocyte cytoplasm. In this respect it is like the limiting membrane of the asexual forms.

In addition to the membrane differences, the mitrochondria of gametocytes are denser and as a rule smaller than those in the trophozoites (Figs. 1, 3). This feature is not invariable since an occasional dense mitochondrion is seen in a trophozoite or schizont, and even less often a "watery" form is seen in a gametocyte. All of the blood forms of the parasite contain pigment granules and probably lipid droplets. Our pictures do not distinguish between these equally dense bodies except that the more angular are assumed to pigment. The pigment in *P. cathemerium* is not as obviously associated with bits of ingested host cytoplasm and with digestion vacuoles as seems to be the case in *P. lophurae* as described by Rudzinska and Trager.

The nuclei in all parasites present much the same appearance. Their nuclear membranes are much less conspicuous than in higher forms and they all contain nucleolar material. As shown

in figure 4, the nucleoli are composed of a cloud of small dense particles which are identical in appearance to the small particulate component of the cytoplasm. The size and density of these particles as well as the staining properties of the parasites permit one to identify them as the ribonucleoprotein granules of Palade (6).

Endoplasmic reticulum in an easily recognizable form is rarely seen in any of the parasites and the profiles illustrated in figure 5 are the best obtained. The paucity of this organelle may be more apparent than real since it is known to be exceedingly labile and the fixative used may be inadequate for its proper demonstration.

Some of the gametocytes display clusters of multi-sized vesicles associated with laminated fine membranes, (Fig. 6). Such an appearance is highly suggestive of a Golgi zone or apparatus. Although there seems little doubt that this feature is the Golgi apparatus, a more elaborate pattern of fine membranes would add certainty to the diagnosis. However, nothing more complicated than figure 6 has been seen in many hundreds of cells. It is curious that cells displaying a Golgi complex are also characterized by having fewer of the fine granules as compared to other parasites. Also, their nuclei tend to be relative larger in relation to the cytoplasm. These features permit the suggestion that gametocytes containing a prominent Golgi apparatus are the microgametocytes and those with abundant granules are the macrogametocytes.

Discussion

Since previous accounts on protozoa have recorded the electron microscope appearance of all the usual organelles of a typical cell, mitochondria and endoplasmic reticulum (8), Golgi appa-

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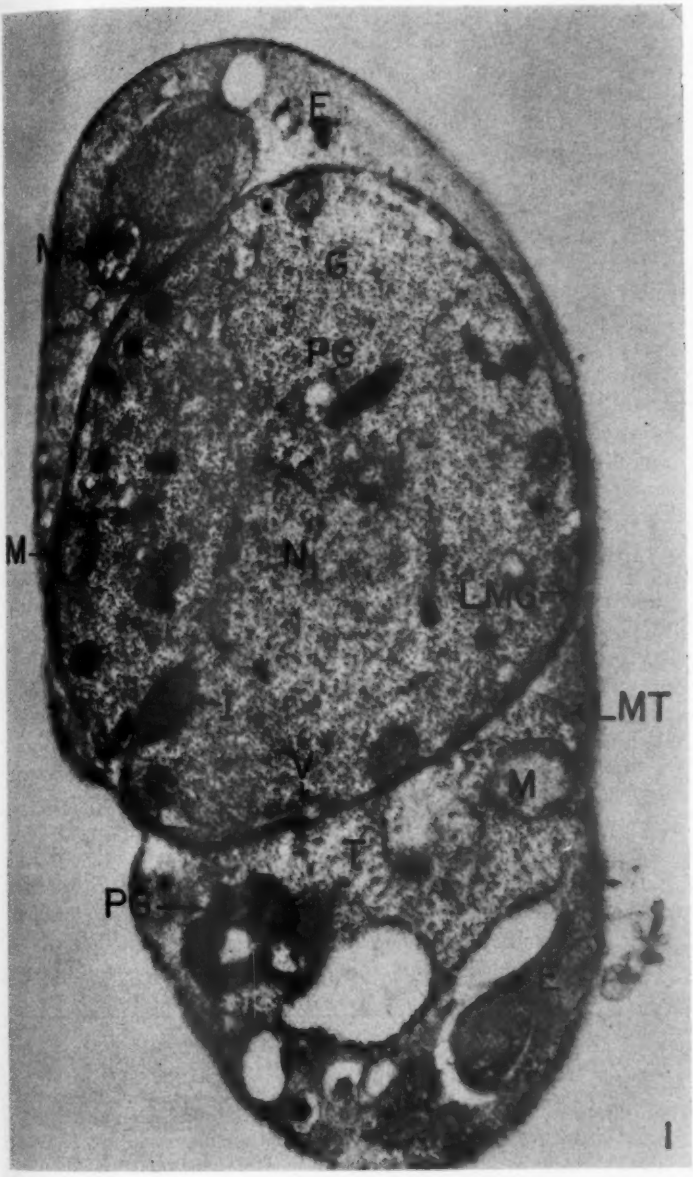
PLATE 1

FIG. 1. A canary erythrocyte (E) containing a gametocyte (G) and a piece of a trophozoite (T). The limiting membrane (LMG) of the gametocyte is thick and prominent while that of the asexual form is thin and inconspicuous (LMT). Mitochondria (M) are prominent features of the erythrocyte, the gametocyte and the trophozoite. Pigment granules (PG) are seen in both parasites. At (I) the limiting membrane of the gametocyte is invaginated and surrounds a mass of erythrocyte cytoplasm. The nucleus (N) of the gametocyte is separated from the cytoplasm by a faint nuclear membrane. A vacuole (V) partially interrupts the limiting membrane. X 25,000.


E
G
PG
M
LMG
LMT
M
PG
I

ratus (2), nucleus and nucleolus (5), there is little that is exceptional about malarial parasites prepared and observed in this manner. Their nuclei seem to lack the elaborate limiting membranes of many free living forms of protozoa (5) and the Golgi apparatus is relatively simple. Likewise, the endoplasmic reticulum is less elaborate and the mitochondria less complex than in many unicellular and multicellular forms. All in all the blood forms of Plasmodium seem to be relatively simple cells from an ultra-structural standpoint.

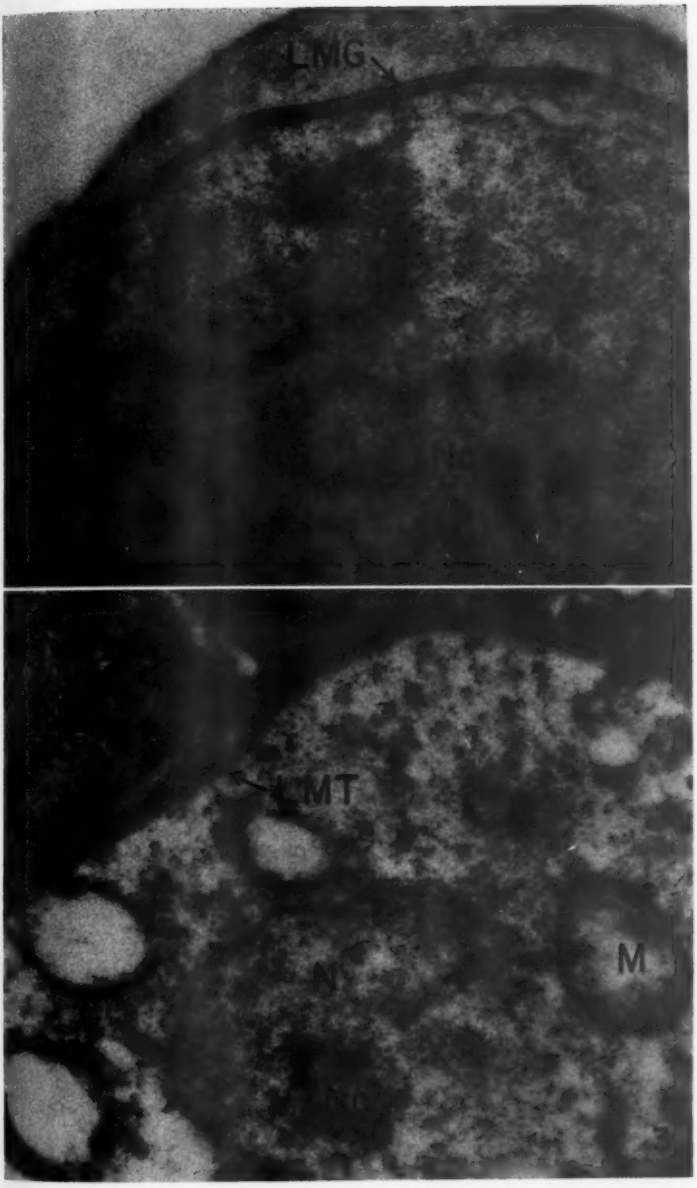
Despite their apparent simplicity, the observed differences between the asexual forms and the gametocytes give some indication of the high degree of specificity that is so clearly evident from the known properties of these cells. The mere fact that the mitochondria in sexual and asexual cells are not alike under identical conditions of fixation is evidence of underlying biochemical and biophysical differences. Most of all, the striking contrasts between the limiting membranes suggest that the exchange of materials between gametocyte and host cell is different than that of the other forms of parasite. Until the gametocyte shown in figure 1 was seen, it seemed plausible to assume that this form did not include engulfment of discernible pieces of erythrocyte cytoplasm as a method of securing material for growth. Since this process does seem to occur the differences occasioned by the two types of membranes must be far more subtle than first supposed, the nature of which we cannot even guess at.

The suggestion that a prominent Golgi apparatus is characteristic of the microgametocyte is in keeping with the prominence of this feature during spermatocyte development in higher forms (1). Mere presence or absence of a well developed Golgi complex might be attributed to the random chances of sectioning if it were not for the accompanying inconspicousness of the fine granular component of the cytoplasm. It is recognized that further study of the *P. cathemerium* and other species as well is needed to con-

PLATE 2

FIG. 2. A portion of a gametocyte to show that the limiting membrane, (LMG) consists of three dense lines separated by lighter intervals, the nucleus (N), nucleolus (Nc) and a mitochondrion (M) are also visible. X 41,000.

FIG. 3. A portion of a trophozoite showing the limiting membrane (LMT), nucleus (N), nucleolus (Nc), and a mitochondrion (M); NE is the nucleus of the host cell. X 41,000.


LMG
LMT
M

firm these suggested distinctions between the male and female gametocyte.

The immediate proximity of acidophilic red cell cytoplasm and basophilic parasite cytoplasm when they are seen in stained preparations, correlates very well with the absence of the small dense particles of Palade in the former and their abundance in the latter. This observation may be noted as further evidence of the soundness of Palade's association of the particles with cytoplasmic basophilia.

Summary

Canary blood infected with *Plasmodium cathemerium* was fixed by injecting it into 2% osmic acid solution, embedded in methacrylate, sectioned with an ultra-microtome, and studied in an electron microscope. In thin sections the gametocytes are easily distinguished from the asexual forms by the presence of a limiting membrane consisting of three distinct dense lines separated by narrow less dense intervals. The limiting membrane of the asexual forms consists of two much thinner dense lines. In addition to the membrane differences, the mitochondria of the gametocytes are smaller and contain a matrix of much higher density. Some of the gametocytes display a prominent Golgi complex; in such cells the small particulate component of the cytoplasm is much less conspicuous. These cells are tentatively identified as the microgametocytes.

REFERENCES AND NOTES

1. Burgos, M. H. and D. W. Fawcett: Studies on the fine structure of the mammalian testis. 1. Differentiation of the spermatids in the cat, *Feliz domestica. J. Biophys. and Biochem. Cytol.* 1: 287–300, 1955.
2. Cohen, A. I.: Electron microscope observations of *Amoeba proteus* in growth and inanition. *J. Biophys. and Biochem. Cytol.* 3: 859–865, 1957.

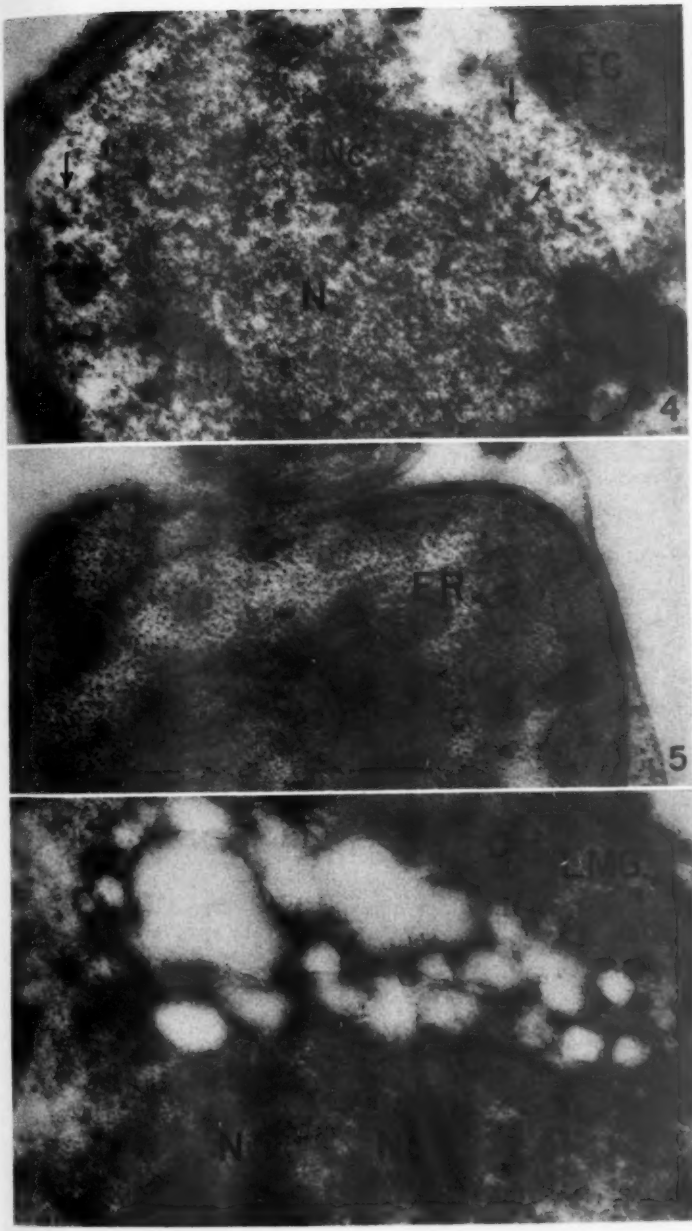
PLATE 3

FIG. 4. This figure illustrates the identity of size and density of the small particles of the nucleolus (Nc) and those of the cytoplasm indicated by arrows, erythrocyte cytoplasm (EC), nucleus of the trophozoite (N). X 50,000.

FIG. 5. A piece of a gametocyte showing indications of endoplasmic reticulum (ER).

FIG. 6. From a gametocyte showing juxtanuclear vesicles and associated fine membranes. X 32,000.

Such an arrangement is characteristic of the Golgi apparatus. (N) nucleus, (Nc) nucleolus, (C) cytoplasm, (LMG) limiting membrane. X 35,000.


EC
MG
N
4
FR
5
MG
N

3. Fulton, J. D. and T. H. Flewitt: The relation of *Plasmodium berghei* and *Plasmodium knowlesi* to their respective red cell hosts. *Trans. Roy. Soc. Trop. Med. and Hygiene.* 50: 150–156, 1956.

4. Goodman, J. E., E. B. Reilly, and R. E. Moore: Electron microscopy of formed elements of normal human blood. *Blood* 12: 428–442, 1957.

5. Harris, P. and T. W. James: Electron microscopic study of the nuclear membrane of *Amoeba proteus* in thin section. *Experimentia* 8: 384–385, 1952.

6. Palade, G. E.: A small particulate component of the cytoplasm. *J. Biophys. and Biochem. Cytol.* 1: 59–68, 1955.

7. Rudzinska, M. A. and W. Trager: Intracellular phagotrophy by malaria parasites: an electron microscope study of *Plasmodium lophurae*. *J. Protozool.* 4: 190–199, 1957.

8. Sedar, A. W. and K. R. Porter: The fine structure of cortical components of *Paramecium multimicronucleatum*. *J. Biophys. and Biochem. Cytol.* 1: 583–604, 1955.

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THE REPRODUCTIVE TRACT OF THE FEMALE NINE-BANDED ARMADILLO

A. C. Enders and G. D. Buchanan*

Introduction

The reproductive system of the nine-banded armadillo has in the past occasioned considerable interest because of two singular features, specific polyembryony (Newman and Patterson, 1909) and delayed implantation (Patterson, 1912). Recently a third unique aspect of reproduction in this species, implantation in ovariectomized females, has been demonstrated (Buchanan *et al*, 1956).

Studies by Newman and Patterson (1910), Patterson (1913), Altmann (1924), Hamlett (1932, 1935) and Newfang (1947) comprise most of the pertinent knowledge of the armadillo reproductive tract. These studies were concerned only incidentally with the morphology of the adult tract; consequently, many histological aspects remain unexplored.

As a part of a general study of the delay of implantation in the armadillo, the female reproductive tract has been examined by several histological and histochemical methods. This work forms a base of reference from which the effects of experimental modification of the reproductive cycle may be gauged.

Materials and Methods

The armadillos used in this work were procured within fifty to one hundred miles of Houston, Texas. Animals which were purchased from suppliers were ordinarily used within a few days of capture. Additional material came from animals shot in the field by the authors.

During the past five years, the reproductive tracts of over two hundred females in various stages of the reproductive cycle have been examined. In the present study, reproductive tracts from immature animals, non-ovulated adult animals, animals in the delay period and implanted animals were used.

Reproductive tracts were dissected out and fixed in Zenker's fluid, Rossman's fluid, Carnoy's fluid, 80% alcohol or 10% formalin. For general histology hematoxylin and eosin preparations were made. Polysaccharides were demonstrated by the Periodic-Acid-Schiff test using material fixed in Rossman's or Carnoy's Fluids. Lipid was demonstrated by the Sudan black method. Cholesterol and/or its esters was shown by the Schultz method. A variation of the

Gomori method for the demonstration of alkaline glycerophosphatase activity was employed.

Description

Gross Relations of the Female Tract. The internal genitalia of the female armadillo lie within the pelvis and consist of the ovaries, oviducts (uterine tubes), simplex uterus, and the vagina. Externally the urogenital canal opens through the prominent vulva.

The bluntly pointed uterus is flattened dorso-ventrally and lies between the rectum and the bladder. In the non-pregnant animal the body of the uterus is at the level of the iliopectineal eminences of the ilium, while the fundus extends somewhat anteriorly. The cervical region of the uterus lies at the level of the acetabula. The long axis of the uterus is oriented anterior-posteriorly except during the latter stages of pregnancy when it is deflected ventrally. The uterine branch of the inferior hypogastric artery courses in part through the broad ligament, reaching the uterus at about the caudal level of the corpus uteri. Caudally this vessel anastomoses with vessels from the bladder.

The round ligaments pass from the junction of the oviducts with the uterus to the inguinal ring. The reflected peritoneum forms the vesico-uterine fold ventrally, and the recto-uterine fold dorsally. The fibrous connective tissue beneath the serosa of these folds forms the dense cardinal ligaments laterally, the sacro-uterine ligaments dorsally, and a tenuous ventral ligament beneath the vesico-uterine fold.

The bean-shaped ovaries lie cranio-lateral and slightly dorsal to the uterus. The peritoneal folds which form the suspensory ligament cranially, and the mesovarium caudally, cover much of the ovary. Nevertheless these rather ill-defined ligaments allow considerable movement of the ovaries, (they can be drawn without stretching about an inch and a half from the pelvic wall). The ovaries are oriented roughly parallel to the long axis of the animal, with the convex, retroperitoneal portion being lateral and the concave free surface being medial.

The infundibulum of the oviduct is attached to the cranial aspect of the concavity of the ovary so that the fimbriated folds are generally situated directly over the concavity. The ampullary portion of the oviduct coils around the cranial pole of the ovary

before coursing posterio-medially along the convex side of the ovary. The isthmic portion extends from the caudal pole of the ovary to the uterus, and is relatively uncoiled. The mesosalpinx unites the oviducts with the mesometrium, and in passing between the ampullary and isthmic portions forms a partial bursa over the ventral aspect of the ovary.

The proper ligament of the ovary arises as a prominently thickened anterior aspect of the mesometrium at the caudal margin of the ovarian concavity. The ovarian artery and vein course through the suspensory ligament and send numerous branches into the convex side of the ovary before anastomosing with the uterine vessels.

Ovaries. The shape of the ovaries is somewhat misleading in that the concavity actually represents the cortex whereas the convexity is the area through which the blood vessels enter the ovary (hilum). Associated with the ovary are various "embryonic remnants"; rete tubules, epoöphoron, medullary cords, and accessory adrenal corticular tissue.

The structure of the epoöphoron is similar to that found in other mammals. These tubules are invariably found at the cranial pole of the ovary, and frequently are found extending all along the convex margin of the ovary. The tubules of the epoöphoron are occasionally confluent with the rete tubules. There do not appear to be any openings into the peritoneum however. The epithelium of the epoöphoron is composed of ciliated, columnar cells, which frequently contain glycogen granules.

The rete tubules (Fig. 5) are usually restricted to the cranial pole of the ovary, being generally located between the epoöphoron and the body of the ovary. Frequently they are continuous with the tubules of the epoöphoron and also with medullary cords. The epithelium of the rete is characteristically low cuboidal. Although a lumen is usually present in the rete, sometimes solid cords are formed. When solid cords are formed, the cells are usually pavement cuboidal.

The medullary cords of the ovary are usually at the cranial pole, but frequently extend further into the ovary, especially over the upper half of the greater curvature. The medullary cords occur in several different forms. The commonest form is simply a group of cuboidal cells in strands or clusters. Strands of cells in solid "tubules," with nuclei located peripherally and

rather indistinct cytoplasmic membranes centrally, are also frequent. Some of the solid "tubules" are similar to those which have been referred to by League and Hartman (1925) as anovular follicles (Fig. 4) and by Wilcox and Mossman (1945) as testicular cords. Additionally, there are occasional large cells within the medullary cords which exhibit mitotic figures. Rounded granular cells resembling the embryonic "interstitial cells" are often associated with the medullary cords (Fig. 7).

In over ninety per cent of the females examined, masses of accessory adrenal corticular tissue were found at the cranial poles of the ovaries. These masses are most frequently found in the connective tissue of the mesovarium, and are, in the majority of instances, superficial to the rete area. Occasionally clusters of such cells are found well within the interfollicular stroma.

The form of the accessory adrenal tissue cells is quite variable. The variation seems to be correlated with the size of the tissue mass rather than with the stage of the reproductive cycle. Small masses of adrenal cells usually contain large cuboidal cells filled with lipid droplets which give a positive Schultz test for cholesterol and/or its esters and positive tests for carbonyl groups (after formalin fixation). Larger masses of accessory tissue exhibit two zones; an outer one of cells like those described above, and an inner zone of smaller cells containing less lipid, and in which the lipid is less easily extracted. Extremely large masses of accessory adrenal corticular tissue have a third zone at the margin of the mass which resembles superficially the zona glomerulosa of the adrenal proper (Fig. 6).

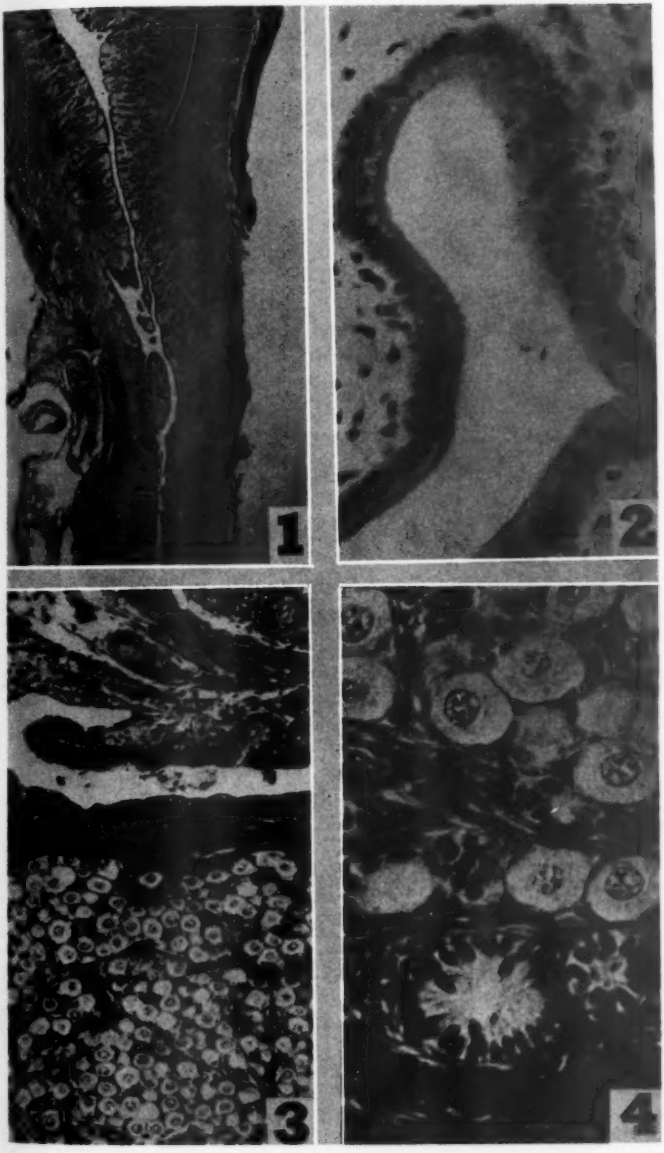
PLATE 1

FIG. 1. Transition from uterine to vaginal mucosa. The lower left is the vesico-uterine fold. At the right the recto-uterine fold has been removed. Typical uterine endometrium is seen in the upper margin of the picture. Vaginal canal is at the lower margin of the picture. H & E. 10 X.

FIG. 2. Vaginal epithelium. The columnar epithelial cells always possess a distal fringe of mucopolysaccharide. H & E. 440 X.

FIG. 3. Cross-section of the concavity of the ovary. In the lower portion of the picture, large masses of ovocytes are seen. A dense tunica albuginea separates these cells from the germinal epithelium. A fimbriated fold of the Fallopian tube is above the germinal epithelium in this illustration. Hematoxylin, Biebrich Scarlet, Orange II, and Fast Green. 110 X.

FIG. 4. Cords of ovocytes. Note the many epithelial cells between the ovocytes, also the "anovular follicle" in the lower portion of the picture. Hematoxylin, Biebrich Scarlet, Orange II, and Fast Green. 440 X.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Because it is largely contained within the folds of the suspensory and mesovarial ligaments, there is very little free surface on the armadillo ovary. A cuboidal germinal epithelium covers the surface of the concavity only. This epithelium is separated from the rest of the ovary by an extremely dense tunica albuginea, beneath which are large numbers of primary ovocytes (Fig. 3). These germ cells radiate in cords into the ovary from the concavity. Numerous small cubodial cells form the other epithelial component of these cords (Fig. 4). Between the cords are the connective tissue elements of the stroma. Occasionally a few small cells which contain lipid droplets are found scattered among the connective tissue elements. The lipid is not extracted by acetone. There are no interstitial gland cells of the type found so commonly in rodents and carnivores.

Primary follicles are most commonly found in or near the cords of germ cells. Secondary follicles and antral follicles are more scattered in position. The zona pellucida can be demonstrated first in the primary follicle and is quite well developed by the antral stage. Follicular development with regard to increase in size and in number of layers of granulosa cells is not particularly unique and has been described by Newman (1912). Consequently, only those points of particular interest which represent either new or conflicting observations are described here. Regarding the latter type of observation it should be pointed out that antrum formation appears to be by secretion, rather than cell breakdown as suggested by Newman.

Since glandular interstitial tissue is lacking in the armadillo

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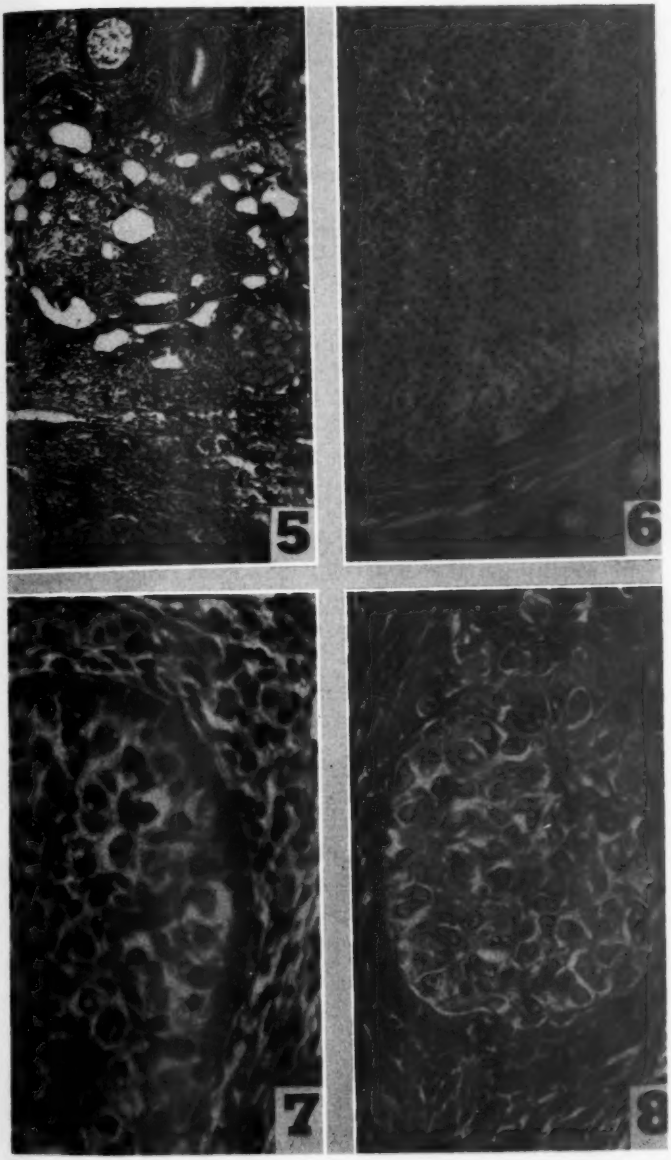
PLATE 2

FIG. 5. Cranial pole of the ovary. In the middle are the rete tubules, in the upper right a tubule of the opoöphoron, and in the right middle of the picture a nodule of adrenal corticular tissue. Hematoxylin and Aniline Blue. 65 X.

FIG. 6. Large nodule of accessory adrenal corticular tissue. Note the apparent formation of zones from the periphery medially. Hematoxylin and Aniline Blue. 110 X.

FIG. 7. "Testis cord". Numerous large cells are in various states of division or pycnosis. At the upper right portion of the figure are seen a few interstitial cells. Hematoxylin, Biebrich Scarlet, Orange II, and Fast Green. 440 X.

FIG. 8. An epithelial mass formed from a degenerating follicle. The basement membrane is still quite distinct around much of the follicle. The mass is formed of granulosa cells. Note that the theca interna cells have already largely disappeared. Hematoxylin, Biebrich Scarlett, Orange II, and Fast Green. 440 X.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ovary, the development and subsequent fate of the glandular theca interna cells is easily followed. The theca interna has from its inception a pronounced alkaline glycerophosphatase activity which persists throughout the life of the follicle. The only structures besides the theca interna with a high phosphatase activity are some of the small blood vessels. Consequently, the demonstration of phosphatase is particularly useful for tracing the history of the theca interna. Epithelioid theca cells are first distinguishable in the solid (secondary) follicle stage. These cells appear to be formed in small clusters from the stromal cells. They are readily distinguished by the presence of lipid droplets.

The masses of epithelioid cells increase within the theca interna during maturation of the follicles. In mature follicles the epithelioid cells are larger than the granulosa cells, and form a continuous layer several cells thick. Numerous fine lipid droplets and a few larger droplets can be demonstrated in the epithelioid cells at this stage. Most of the droplets can be extracted with acetone and are also Schultz positive.

With the onset of follicular atresia the cells of the theca interna usually atrophy. Frequently the theca interna atrophies before atrophy of the granulosa cells has progressed beyond the initial stages. Occasionally a few epithelioid cells from the theca interna persist within the connective tissue stroma for an appreciable time. In this case the remaining cells are small, scattered, and contain only a few lipid droplets which are not extracted by acetone.

Degenerative changes can occur at any stage of the follicular cycle. Developing ova normally contain appreciable glycogen but little demonstrable lipid. However, with the onset of atresia, the glycogen granulation becomes coarse and numerous lipid droplets accumulate. The presence of many large, irregular lipid droplets within the ova is a sign of degeneration which can be observed in all stages of primary ovocytes from the germ cell strands to mature follicles.

Lipid droplets comparable to those found in the epithelioid theca cells do not appear in the granulosa until antrum formation. The droplets are not very numerous and remain until ovulation unless atresia occurs. The formation of rather large fat droplets within the granulosa cells indicates follicular atresia. The sequence of degeneration of the ovum, granulosa and theca

interna is variable in different follicles. However, the granulosa is usually the last element to lose its integrity. (The zona pellucida is apparently very resistant for it persists long after the other structures of the follicle have disappeared.) When large antral follicles undergo atresia, the granulosa cells usually become necrotic and subsequently disintegrate without previous hypertrophy. However, a second type of atresia is more common, especially in pregnant animals. When the larger solid follicles, or smaller antral follicles, become atretic in early pregnancy, the granulosa cells hypertrophy while the other elements degenerate (Fig. 8). Thus numerous masses consisting of large, irregular granulosa cells can be found in ovaries of the delay period. Such masses of cells resemble superficially the "interstitial glands" of other forms, but do not contain lipid droplets. Accessory corpora lutea or corpora atretica are extremely rare and are quite distinct from the degeneration bodies formed by the hypertrophied granulosa cells.

Normal ovulatory stages have been observed in only four animals. However, ovulation has been induced by the injection of pregnant mare serum followed by chorionic gonadatropin in six other cases. Thus our material does not permit extensive description. Formation of the secondary follicular fluid and streaming of the granulosa cells precedes ovulation which is generally accompanied by considerable hemorrhage. Following the subsequent vascular invasion of the granulosa, the granulosa cells rapidly luteinize. Large, fully luteinized cells are found well before the end of the first two weeks of the delay period. At the margins of a developing corpus luteum from an animal in which an eight cells stage ovum was discovered, there were small masses of theca lutein cells. These cells were much smaller than the luteinizing granulosa cells. Follicle rupture always occurs at the concavity of the ovary, but the subsequent growth of the corpus luteum is toward the convex surface.

The corpus luteum of delay is large constituting more than half of the total volume of the ovary. The luteal cells are large and irregular in outline. However, only one type of luteal cell can be distinguished. Lipid droplets are very rare in the luteal cells, but when present are positive with the Schultz test and carbonyl tests (after formalin fixation). Mitochondria are large and extremely numerous. Glycogen granules are common and

become more abundant after implantation. When the corpus begins to degenerate in late pregnancy, non-acetone-extractable lipid begins to accumulate.

The stroma of the corpus luteum consists of fibroblasts, a few collagen and numerous reticular fibers, and an extensive system of sinusoids. The luteal cells are intimately associated with the sinusoids. Occasionally a single luteal cell will largely surround one sinusoid and also be in contact with several others.

Oviduct. Fimbriated folds of the infundibulum converge to form a normally narrow passage into the ampullary portion of the oviduct. This portion is relatively glandular with a rather broad lumen. The epithelium forms numerous folds, the basal portions of which are less dilated and more tortuous forming basal glands. Scattered smooth muscle bundles are present in the ampulla through most of its length.

The transition to isthmic portion of the oviduct occurs at about mid-ovary. In the isthmus, smooth muscle forms a complete, fairly thick circular layer and the lumen becomes relatively obscure. The epithelium becomes reduced to a luminal layer with three or four primary folds. Occasional minor folds form intraepithelial glands.

The epithelium of the oviduct enters the myometrium as an undulating line of largely ciliated columnar epithelial cells, with occasional pockets, and areas of pseudostratification. The oviduct opens into the lumen of the uterus in the cranial portion of the lateral fold. The uterine glands and luminal epithelium in this region are more highly ciliated than in other portions of the uterus.

The epithelial cells of the infundibulum and ampulla are most highly developed during the late ovulation and delay periods. Three cell types are commonly observed. These are ciliated cells, secretory cells, and a third cell type which may represent either juvenile cells or exhausted secretory cells. Mitotic figures are not abundant during delay. The lumen of the ampullary portion of the oviduct is dilated, but the lumina of the basal twists of the glands are not. Ciliated cells constitute somewhat less than 50% of the total. In material fixed in Flemming's fluid, the nuclei are plump and the majority of the cells tall columnar to stentor shaped. The greater shrinkage produced by other fixatives (e.g.,

Zenker's) results in cellular extrusion, especially of the secretory cells.

The distribution of lipid droplets within the epithelial cells is scattered and does not appear to be confined to any one cell type. When cell droplets are present they are usually in a basal position though occasional distal droplets may be seen. Glycogen is present in all of the luminal epithelial cells during the delay period. It is especially abundant in the fimbriated folds. There is less glycogen in the basilar twists of the glands.

The oviductal epithelium of animals at other stages of the reproductive cycle is less hypertrophied. Glycogen is much reduced at other stages and PAS positive material is largely restricted to the luminal fringe and basement membrane. Lipid distribution shows little variation with reproductive state. The nature of the secretion product of the armadillo oviduct has not been elucidated, but it should be noted that it does not have the characteristics of mucous, and that the blastocyst arrives in the uterus without any extracellular coats.

The Uterus. There are three regions of the uterus: the fundus which extends from the cranial tip to the level of the entrance of the oviducts, the corpus uteri which comprises the region from the oviducts to about the recto-uterine fold, and the cervix which is marked externally by constriction of the uterus slightly below the ligamenta teres and the vesico-uterine fold.

In sub-adult and virgin non-ovulated adult animals, the uterus measures about 14 mm. in length, 10 mm. in breadth, 7 mm. in depth, and weighs approximately 550 mg. During the period of delay of implantation, the uterus measures about 18 mm. by 12 mm. by 9 mm. and weighs about 1120 mg. The uteri of nonovulated multiparous animals are similar in size to those of delay animals.

The histology and histochemistry of the uterus in delay and implanted animals has been described in detail in another paper (Enders *et al.*, 1958) and will be mentioned here only for purposes of comparison.

The striking feature of the endometrium is the extensive layer of large anastomotic sinusoids. These sinusoids which are quite large in adult animals, divide the endometrium into basal, sinusoidal and luminal regions. The sinusoids are larger in the fundic

area of the uterus and the luminal region is much reduced in thickness.

The stroma supporting the tubular endometrial glands is made up of areolar connective tissue. In immature animals the connective tissue is dense and cellular rather than fibrous. The nuclei are similar in size to the nuclei of the epithelial cells. The stroma of adult nonovulated animals is less dense and the nuclei appear more spindle-shaped and lie perpendicular to the luminal surface. Multiparous animals and animals in the period of delay of implantation have a relatively sparse stroma supporting the glands in the fundic area. The stroma of the cervix is quite dense and fibrous composing the largest part of the mucosa.

The blood pattern of the endometrium, which is so marked in delay animals, is scarcely seen in immature animals, first becoming apparent in adult nonovulated animals. The sinusoids are fed by irregularly coiled arterioles which enter from the myometrium, course toward the luminal surface and typically loop back to the intermediate zone and enter the sinusoids.

The stroma of the endometrium is mildly positive after the PAS procedure whether or not this procedure has been preceded by diastase digestion. It is mildly acidophilic at a pH of 5.7. In the area of the sinusoids the stroma displays a moderate alkaline phosphatase activity.

The glands of the corpus uteri are moderately coiled and frequently branched in immature animals. The cells are low columnar with oval basally situated nuclei. In adult nonovulated animals the glands are more elongate, less tortuous and the cells are somewhat taller. In delay animals the glands are essentially straight and tubular and are slightly dilated. The cells are columnar with oval basally situated nuclei, although in the basal portion they are low columnar and the nuclei tend to be more spherical. The cells of the sinusoidal portion are relatively more vacuolated than elsewhere. The gland cells are frequently ciliated, especially near the oviducts although the cilia are inconspicuous in immature animals.

The fundic glands appear to have no region comparable to the neck region of the glands of the corpus uteri, their proximal portions resembling the sinusoidal portion of the corpus uteri glands. There is a transition to less elongate and uncoiled glands in the cervical region.

Immature animals show small amounts of glycogen in the proximal cytoplasm of the cells of the basal twists of the glands. This amount increases slightly in adult animals and in ovulated animals moderate amounts of glycogen are present in the cells of both the basal and sinusoidal regions. Glycogen increases after implantation.

Small amounts of lipid are present in the cells of the basal region of the body glands in immature and adult animals. Delay animals show slight amounts in the superficial portions of the glands also. Large amounts of lipid are found in the cells of the fundal glands in immature animals but this decreases following implantation.

The luminal epithelium of immature animals is simple cuboidal to columnar with the crowding of the cells being variable. In adult animals the cells are columnar and fairly close-packed. During the delay period the cells become very crowded and range from pseudo-stratified to palisade to stratified. A definite fringe of mucopolysaccharide is present on the distal surface of the cells of adult and delay animals. Some glycogen is found but it is not uniform.

The myometrium is quite thick in immature armadillos being often more than three times the thickness of the endometrium. This relative size decreases in older animals and the myometrium of ovulated animals is only slightly thicker than the endometrial layer. The myometrium contains moderate amounts of glycogen at all stages.

Three layers are distinguishable in the myometrium. The outer layer consists of large layers of smooth muscle which tend to be oblique or longitudinal in the peripheral portion and circular in the inner portion.

The middle area, the area vasculosa, has relatively smaller bundles of smooth muscle surrounding the blood vessels. The blood vessels lie fairly deep as they enter from the broad ligaments but are superficial in the mid-dorsal and mid-ventral areas.

The inner myometrial layer is composed of a network of small smooth muscle bundles. In immature animals the fibers at the myometrial-endometrial junction lie parallel to the luminal surface so that the junction is quite distinct. This junction becomes less distinct in adult animals. Lying between the meshes of

muscle bundles are a series of sinusoids which anastomose freely and connect with endometrial sinusoids as well as the veins of the area vasculosa. These sinusoids are less pronounced in immature animals and are frequently difficult to distinguish. In adult animals they are more apparent due to the thinning of the connective tissue and are quite distinct in multiparous animals.

The Vagina. The lower portion of the urogenital system may be described as consisting of three tubes. From the external orifice, the common urogenital sinus courses anteriorly for slightly more than 3 cm. At this point, it is joined to two tubes, a ventral tube, the urethra, which leads to the bladder, and a dorsal tube, the vagina, which leads to the uterus. These latter tubes are about 3 cm. in length. The two tubes are contiguous for most of their length and are invested in a common connective tissue sheath. Unless this common sheath is carefully dissected apart the bladder and uterus appear to enter the urogenital sinus directly.

The vagina is normally rather thin-walled, flaccid and distensible. It is limited at its anterior end where it adjoins the uterus by a thickening of the submucosal connective tissue. At its junction with the common urogenital sinus, the vagina protrudes slightly into the common sinus. Its orifice (within the common sinus) is partially surrounded by a semilunar lip, which in fresh material is slightly turgid. The lip appears to serve to prevent the entry of urine into the vagina.

At the transition to the vagina from the cervix (Fig. 1) there is an increase in the amount of connective tissue of the muscularis, especially in the outer layers. Some of the collagen fibers of the outermost layer of the muscularis appear to be reflected dorsally into the recto-uterine fold. There is a diminution in width of the innermost muscle layer also.

The vagina is lined with a tall columnar epithelium which bears a prominent distal fringe of mucus (Fig. 2). Shallow epithelial glands occur throughout the length of the tube. The cells which comprise the glands are similar to those lining the lumen. The lip which guards the distal end of the vagina is histologically similar to the luminal epithelium. In hematoxylin and eosin stained preparations, the uterine epithelium and that of the vagina appear to blend gradually, however, the two can easily be distinguished. In sections subjected to the Periodic-Acid-Schiff

method the change is very sharp due to the sudden acquisition of a distal fringe of mucus by the vaginal cells.

In an effort to determine the reproductive status of individual animals, vaginal smears were taken. The nature of the epithelium precluded the sloughing off of cells but it was thought that leucocytic invasion might be found during periods of anestrus or diestrus. Unfortunately, no intelligible pattern was seen in the smears taken although occasionally smears containing appreciable numbers of leucocytes were found.

Discussion

The ovary of the armadillo apparently undergoes considerable change in external form from the late foetal stage to the adult condition. In the late foetus, according to Newfang (1947) the cortex occupies the greater part of the ovary and is convex. The subsequent growth of the ovary is limited in the area of the cortex resulting in the formation of the concavity normally found at this position. The thickness of the tunica albuginea and the lack of germ cells observed within this structure indicate, as Hamlett (1935) has pointed out, that it is highly improbable that germ cells are formed from the germinal epithelium in the adult. The vast number of ovocytes in the corticular masses of the adult ovary are grossly in excess of the numbers which would be incorporated into follicles of all types throughout the life of the animal. Examination of the cords of ovocytes and associated cuboidal epithelial cells reveals that the area is a center for differentiation of germ cells and for the multiplication and differentiation of granulosa cells. However, the absence of a series of initial stages of germ cell development in this area indicates that this region is probably not a center of multiplication of germ cells in the adult animal.

The hermaphroditic nature of the foetal armadillo ovary has been noted by Newfang (1947). It is interesting that such male features as rete tubules connecting with the epoöphoron and with medullary cords persist in the adult ovary. League and Hartman (1925) mention the presence of anovular follicles in the armadillo ovary. Wilcox and Mossman (1945) speculate that what the above authors observed were in reality "testis" cords. It has been

our observation that while there are a few anovular follicles in the region of the cortex of the ovary, the solid "tubules" found elsewhere represent modifications of the medullary cords and are thus "testis" cords in the sense referred to by Wilcox and Mossman (1945). The occasional mitotic figures which have been observed in the medullary cords appear in the cords of the solid type.

The significance of the accessory adrenal corticular tissue is enigmatic. It is almost universally found, but the total quantity present in any one animal varies widely. It does not appear to be present (at least in the form found in the adult) in the neonatal animal. The histochemical and histological characteristics of the accessory corticular cells are similar to those of the normal adrenal cortex. It is doubtful, however, that the accessory tissue constitutes a quantitatively significant contribution to the total mass of adrenal tissue.

Although the corpus luteum of delayed implantation does not contain much lipid, it is histologically a fully luteinized structure. Furthermore, Talmage *et al* (1954) have demonstrated that there is a high level of progesterone in the serum of delay animals. Hamlett (1932), on the other hand, thought that the corpus only became active when it became vacuolated at the onset of implantation. A search of our material does not reveal much increase in vacuolation at nidation. It does, however, reveal that there is frequently an increase in the quantity of glycogen found in the luteal cells after implantation which might cause vacuolation with some fixation procedures.

There is some problem in deciding whether the tubal connection between the body of the uterus and the urogenital sinus should be designated a vagina. The vagina, in those mammals in which it has been described, differs from the rest of the Mullerian system in that it is lined with a stratified epithelium at least during some stages of the reproductive cycle. The stratification probably protects the vagina during its function of ensheathing the penis during intromission. Witchi (1959) points out that there is considerable divergence of opinion regarding the origin of the stratified epithelium. Some workers hold that the primordial (Mullerian) epithelium is supplanted by the sino-vaginal bulbs; others feel there is an invasion of cloacal epithelium; and others

believe the Mullerian epithelium becomes stratified. At any rate stratification does not occur in the armadillo.

Most workers have referred to the dorsal tube as vagina (Owen, 1831; Newman and Patterson, 1910; Patterson, 1912; Witchi, 1959). Since most early works were not histological, it is probable that the tube was so named because it differed grossly from the body of the uterus and was distal to it. Altmann (1924) studied the closely related *Dasypus septemcinctus*, which appears to be like *D. novemcinctus*. He calls the dorsal tube cervix because of the nature of its appearance in histological section, although he points out that it is where the vagina would be expected to be. Altmann also examined a single foetal *D. novemcinctus* and referred to the tube as cervix there also. Newfang in her embryological study states that the armadillo has no vagina but rather that the uterus enters "a dorsal recess of the relatively long ((urogenital) sinus."

Histologically the dorsal tube resembles cervical tissue in that it has a columnar mucus secreting epithelium and is not stratified; however, on the basis of gross structure it does not qualify as a cervix since the lower portion of the body of the uterus, where the tube adjoins the uterus, acts as a restriction to expansion and serves adequately as a cervix. This point is easily verifiable in dissection of the gravid uterus.

From the standpoint of function, it is felt that the dorsal tube may serve as a vagina and receive the seminal discharge. Normally the lip of the tube serves to prevent the entrance of urine and it would seem that the chances of sperm entering the uterus would be much enhanced if they were deposited behind the lips of the tube rather than in the urogenital sinus. Additionally the phallus of the male is of a relatively uniform diameter down to the end of the prominences of the corpora cavernosa. Beyond that point the phallus is much smaller. In viewing the genital tracts of both sexes it is difficult to avoid the conclusion that this distal portion of the phallus enters the dorsal tube.

Summary

A study was made of the female reproductive tract of the armadillo. The ovaries, oviducts, uterus and vagina of adult animals were examined by histological and histochemical methods. The

morphological relations and histological variation of these structures are described and some of the more unusual aspects are discussed.

REFERENCES AND NOTES

Altmann, F.: Beitrage zur Anatomie des weiblichen Genetales der Dasypodiden. Zs. ges. *Anat. Munchen.* 72: 390–406, 1924.

Buchanan, G. D., A. C. Enders and R. V. Talmage: Implantation in armadillos ovariectomized during the period of delayed implantation. *J. Endocrin.* 14: 121–128, 1956.

Enders, A. C., G. D. Buchanan and R. V. Talmage: Histological and histochemical observations on the armadillo uterus during the delayed and post implantation periods. *Anat. Rec.* 130(4): 639–657, 1958.

Hamlett, G. W. D.: The reproductive cycle in the armadillo. *Zeit. f. Wiss. Zool.* 141: 143–157, 1932.

————: Delayed implantation and discontinuous development in the mammals. *Quart. Rev. Biol.* 10: 432–447, 1935.

League, Bessie, and C. G. Hartman: Anovular graafian follicles in mammalian ovaries. *Anat. Rec.* 30: 1–13, 1925.

Newfang, D. M.: Sex differentiation in the nine-banded armadillo, *Dasypus novemcinctus. J. Morph.* 81: 283–316, 1947.

Newman, H. H.: The ovum of the nine-banded armadillo: growth of the ovocytes, maturation and fertilization. *Biol. Bull.* 23: 100–140, 1912.

Newman, H. H., and J. T. Patterson: A case of normal identical quadruplets in the armadillo and its bearing on the problem of identical twins and sex determination. *Biol. Bull.* 17: 181–187, 1909.

————: The development of the nine-banded armadillo from primitive streak to birth, with special reference to the question of specific polyembryony. *J. Morph.* 21: 359–423, 1910.

Owen, R.: Notes on the anatomy of the nine-banded armadillo (*Dasypus peba*, Desm). *P.Z.S.* 1831: 141–144, 1831.

Patterson, J. T.: A preliminary report on the demonstration of polyembryonic development in the armadillo (*T. novemcinctus*). *Anat. Anz., Jena.* 41: 369–381, 1912.

————: Polyembryonic development in *Tatusia novemcinctus. J. Morph.* 24: 559–683, 1913.

Talmage, R. V., G. D. Buchanan, F. W. Kraintz, E. A. Lazo-Wasem and M. X. Zarrow: The presence of a functional corpus lutem during delayed implantation in the armadillo. *J. Endrocrin.* 11: 44–49, 1954.

Wilcox, D. E. and H. W. Mossman: The common occurrence of "testis" cords in the ovaries of a shrew (*Sorex vagrans*, Baird). *Anat. Rec.* 92: 183–195, 1945.

Witchi, E.: Embryology of the uterus: normal and experimental. *Annals of the N. Y. Acad. Sci.* 75: 412–435, 1959.

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THE EFFECT OF OXYPSORALEN UPON LEUKEMOGENESIS IN MICE BY X-RAYS AND METHYLCHOLANTHRENE

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The substance, 8-methoxypsoralen (8-MP) is well known as a photosensitizing compound. Oral administration enhances pigmentation in human skin following exposure to sunlight (1) and (2). When 8-MP is given in the diet, the incidence of ear tumors in Swiss mice exposed to ultraviolet irradiation is markedly reduced. Oxypsoralen also appears to protect against the induction of skin tumors in mice by ultraviolet rays (3).

8-MP was administered orally to C57BL strain mice given whole body x-irradiation to determine whether this compound would inhibit the induction of leukemia by x-rays in mice. Another study was carried out to test whether 8-MP would affect leukemogenesis by methylcholonthrene (MCA) in male mice of DBA/2 strain.

Materials and Methods

Pure C57BL strain mice and DBA/2 male mice used in this experiment have been inbred in this laboratory by brother-sister matings. Animals were treated from 6 weeks of age. 8-MP[c] was added to the diet at the beginning of x-ray or MCA treatment. The mice were fed with a diet consisting of half a gram of 8-MP adedd to each kilogram of ground Puria Laboratory Chow. The administration of 8-MP in the diet was discontinued after ten months.

C57BL strain mice received whole body x-irradiation in eleven doses at four-day intervals, each dose consisting of 80 r[1]. C57BL mice are known to be a low-leukemia strain and to be susceptible to the induction of leukemia by x-ray.

DBA/2 male mice were treated with urethan and painted with MCA. 0.25 per cent solution of MCA in benzene was applied three times weekly, each time to a different site of the skin. Each mouse received a total of 18 paintings. Urethan, found to be a co-leukemogenic agent (4) was injected intraperitoneally, 1.0 mg./gm of body weight. Each mouse received a total of four injections of urethan, given every four days. DBA/2 strain mice are highly susceptible to the induction of leukemia by MCA.

The experimental animals were divided into groups as follows:

Series I: Whole body x-irradiation (C57BL mice)

Group 1—X-rays only

Group 2—X-rays plus 8-MP in the diet

Group 3—8-MP in the diet only
Series II: MCA painting (DBA/2 male mice)
Group 1—MCA and urethan
Group 2—MCA and urethan plus 8-MP in the diet
Group 3—8-MP in the diet only

In both series, groups 2 and 3 have now been observed for about eighteen months after x-ray or MCA treatment. There was no evidence of weight loss in mice by the oral administration of 8-MP during the period of observation.

Results and Discussion. The incidence of x-ray-induced leukemia in C57BL mice is shown in Table 1. A significant difference in the incidence of leukemia was found between group 1 and group 2. Of the mice which received whole body x-irradiation 72.2 per cent developed leukemia within ten months after irradiation.

TABLE 1

The Incidence of Leukemia in C57BL Strain

Group	Treatment	No. Mice Sex		Total	No. Develop. Leukemia Sex		Total	Per Cent
1	Whole body x-rad.	F	9	18	F	8	13	72.2
	(80 r × 11)	M	9		M	5		
2	Whole body x-rad.	F	10	20	F	1	1	5.0
	(80 r × 11)	M	10		M	0		
	Plus Dietary 8-MP							
3	Dietary 8-MP	F	8	18	F	0	0	0
		M	10		M	0		

When C57BL mice were given dietary 8-MP, the leukemogenic effect of x-irradiation was significantly reduced. No leukemia developed in mice receiving 8-MP only. Data suggest that 8-MP inhibited leukemogenesis by x-ray in C57BL mice.

Table 2 shows the incidence of leukemia in DBA/2 male mice which were skin-painted with MCA. There was no difference in the incidence of leukemia between the group treated with MCA and the group treated with MCA plus 8-MP. The incidence of leukemia in MCA-treated mice was 55.2 per cent. However, in mice receiving 8-MP in addition to MCA, nine out of 15 mice developed leukemia, an incidence of 60 per cent. No leukemia was induced in mice receiving only dietary 8-MP. Thus, 8-MP seemed to have no protective effect against the induction of leukemia in DBA/2 male mice by MCA.

TABLE 2

The Incidence of Leukemia in DBA/2 Male Mice

Group	Treatment	No. Mice	No. Develop. Leukemia	Per Cent
1	MCA × 18 and urethan × 4	29	16	55.2
2	MCA × 18 and urethan × 4, plus dietary 8-MP	15	9	60.0
3	Dietary 8-MP	11	0	0

MCA is also carcinogenic in mice when painted on the skin. The incidence of skin tumors in DBA/2 male mice receiving 8-MP orally in addition to MCA painting with less than that in mice treated with MCA only, is shown in Table 3. However, the difference was not statistically significant. It is to be pointed out that skin tumors and leukemia did not co-exist. The skin tumors appeared only in mice which did not develop leukemia. It was not possible to estimate what the incidence of skin tumors might have been among mice which developed leukemia.

TABLE 3

The Incidence of Skin Tumors in DBA/2 Male Mice

Treatment	No. Mice	No. Develop. Skin Tumors	Per Cent
MCA × 18 and urethan × 4	29	7	24.1
MCA × 18 and urethan × 4, plus dietary 8-MP	15	2	13.3

In this study, the oral administration of 8-MP did not affect the induction of skin tumor by MCA in DBA/2 male mice, although 8-MP has inhibited the ultraviolet irradiation-induced skin carcinogenesis in Swiss mice (3).

Summary

1. The effect of 8-methoxypsoralen upon leukemogenesis in mice by x-rays and methylcholanthrene has been studied.
2. The leukemogenic effect of x-irradiation in C57BL mice receiving 8-methoxypsoralen was significantly reduced. However, this compound did not protect against the induction by methylcholanthrene of leukemia in CBA/2 male mice.

3. The oral administration of 8-methoxypsoralen did not inhibit the induction of skin carcinogenesis by methylcholanthrene in DBA/2 male mice.

REFERENCES AND NOTES

1. Fitzpatrick, T. B., Hopkins, C. E., Blickenstaff, D. D. and Swift, S.: Augmented pigmentation and other responses of normal human skin to solar radiation following oral administration of 8-methoxypsoralen. *J. Invest. Dermatol.*, 25: 187–190, 1955.
2. Psoralens and Radiant Energy. Supplement to February, 1959, issue of *J. Invest. Dermatol.*, 32: 363–372, 1959.
3. O'Neal, M. A. and Griffin, A. C.: The effect of oxypsoralen upon ultraviolet carcinogenesis in albino mice. *Cancer Res.*, 17: 911–916, 1957.
4. Kawamoto, S., Ida, N., Kirschbaum, A. and Taylor G.: Urethan and leukemogenesis in mice. *Cancer Res.*, 18: 725–729, 1958.

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[b] Deceased.

[c] 8-MP used in this investigation was kindly supplied by R. A. Hakim, Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

[1] Physical factor: 140 KVP. 5 MA 2 × 1.0 mm AL added filter, 30 cm. target mouse distance. Output 58.8 r per minute.

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ON THE CHROMOSOME NUMBERS OF HUMAN AMNION CELLS IN PRIMARY AND STRAIN CULTURES*

Y. H. NAKANISHI[a], M. V. FERNANDES[b], M. MIZUTANI[c] AND C. M. POMERAT

Cell cultures from human amnion recently have attracted considerable attention. The report of the establishment of a cell which persisted as an easily manageable "strain" by Fogh and Lund (1957) was followed by descriptions of morphological events occurring in the course of the formation of other lines by Dunnebacke (1956). Fernandes (1958) launched a similar line with the important features that within 35 days it appeared to have acquired the rapidly proliferative and nutritionally facultative characteristics which are generally ascribed to this class of cells.

Amnion cell strains have been reported to be particularly useful for virological studies. Pomerat et al. (1958) suggested that giant cell formation in amnion and other cell lines as a result of irradiation might be related to the elevated number of chromosomes typical for some of the cells in this organ.

The present study was undertaken with the hope of providing a more detailed chromosomal analysis of primary and strain cultures of a human amnion than those reported in the previous papers (Fernandes, 1958; Pomerat et al., 1958) with a description of the morphology of their chromosomes.

Materials and Methods

Placental tissue from a healthy woman was obtained from the John Sealy Hospital in Galveston, Texas.

Test objects consisted of 3 types of amnion derivatives:

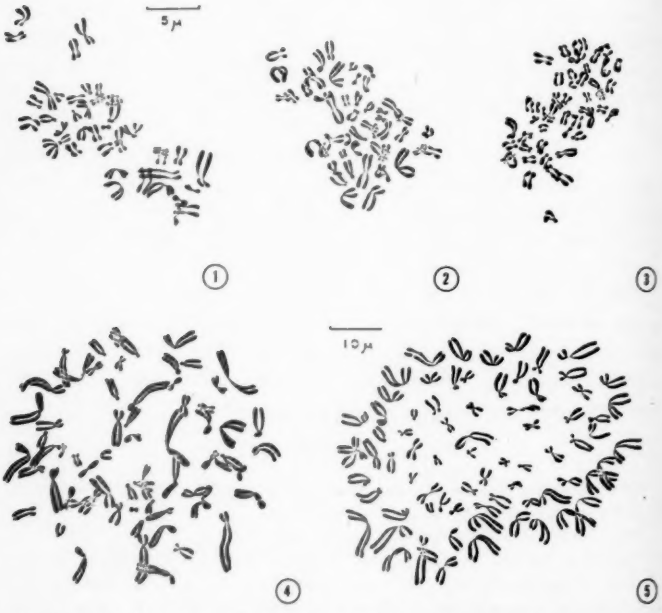
I. *Freshly isolated elements before culture* (cf., figs. 1–3). Using amnion obtained immediately upon delivery, about 150 fragments obtained from various areas measuring approximately 1 mm. on a side were employed for making crush preparations stained with acetic orcein after treatment with a hypotonic solution (1 part of Gey's balanced salt solution plus 9 parts of ordinary tap water) for one hour.

II. *Epithelial cells in primary cultures of amnion tissue.* After 25 days of incubation, cells on cover glasses in roller tubes which had not been freed

with trypsin solution were treated with a hypotonic solution for 10 minutes, fixed with methyl alcohol and stained according to Jacobson's method (Hsu and Pomerat, 1953; Nakanishi, in press).

III. *The development of the Fernandes amnion strain of epithelial cells* (cf., figs. 4–5). This resulted from the trypsinization of amnion tissue and its cultivation without embryonic extract (Fernandes, 1958; Pomerat et al., 1958). On the 35th day from the date of the original trypsinization the chromosome numbers were counted in Rose chamber cultures of the 2nd passage using the same method as that applied for primary cultures. Later a newer technique as described by Hsu and Klatt (1958), Nakanishi and Mizutani (1959) and Nakanishi (in press) was utilized to observed the chromosomes of this strain.

Eagle's medium was employed with the addition of 10 per cent horse serum for primary cultures and for the establishment and maintenance of the strain. At the present time this strain is in the 97th passage, which is over two years after the date of the original trypsinization (April 1957 to May 1959).



FIGS. 1 to 5. Camera lucida drawings of the chromosomes of human amnion cells. FIGS. 1 to 3—freshly isolated cells before culture (42, 48 and 55 chromosomes, respectively). FIGS. 4 to 5—chromosomes of the strain cultures (67 chromosomes in the 2nd subculture and 75 elements in the 84th subculture). The magnification for FIGS. 1 to 4 is given by the scale shown in figure 1 and for figure 5 as shown.

Results and Discussion

I. *The chromosomes of freshly isolated amnion cells.* Walker (1958) found diploid, tetraploid and octoploid numbers of chromosomes in the transitional epithelium of the bladder of mice. For the bladder of newborn mice in which there was a wide range of nuclear size, he reported that out of 139 cells 68 were diploid, 63 were tetraploid and 8 were octoploid. It was concluded that polyploidy and large nuclei in newborn mice were related to normal differentiation by means of which "dome" cells in the surface layer of the transitional epithelium were produced. On the basis of both chromosome counts and modifications of nuclear size, Sachs and Shelesnyak (1955) studied the occurrence of polyploidy in the development of the deciduomata and its absence when this organ was suppressed byan antihistaminic agent. These authors concluded that polyploidy might be related to some specialized activity in the maternal placenta and that since these cells could not reproduce themselves normally or persist, this might provide a possible basis for the limited life of the deciduoma.

In a cytological study of the regenerating rat liver, Makino and Tanaka (1953) and Tanaka (1953) found that while there was a wide variation in the chromosome number of regenerating liver cells from adult rats during restoration after partial extirpation, the most frequent numbers fell in three ranges: diploid (36 to 46 chromosomes), triploid (59 to 68 chromosomes) and tetraploid (78 to 89 chromosomes). However, those in the diploid range were the most numerous.

In order to check the possibility that some areas of the human amnion normally contain cells with more than the diploid number, it was necessary to establish the chromosome count for freshly isolated elements before culture. Unfortunately, the amnion cells obtained directly from fetal membranes showed very little divisional activity. Only 3 cells revealed chromosomes sufficiently well spread to permit accurate counting. The values obtained were 42, 48 and 55 (Figures 1, 2, 3). Even though the chromosomes of only 3 cells were counted, this observation was suggestive of a diploid range for the human amnion.

II. *The chromosomal status of cells in primary cultures.* The chromosome numbers counted in both primary and strain cultures are presented in figure 6. Results for the 24 cells with well-

spread chromosomes obtained in material fixed on the 25th day of *in vitro* life showed a range from 41 to 52 with the highest values at 46 (25%) and 48 (21%).

Since Painter (1923) reported that the chromosome number in man was 48 and Oguma and Kihara (1923) stated that there were 47 chromosomes in the male, a number of workers have challenged these counts. The difficulties encountered in the enumeration of human chromosomes are obviously due mainly to the techniques associated with the handling of cells containing large numbers of these structures. The development of methods for the spreading of chromosomes in cell culture has made possible more critical studies of their number and morphology. Hsu (1952) reported 48 chromosomes in the material from tissue culture. However, he now states (Hsu, 1959) that even with hypotonic solution treatment which greatly enhances the spreading of the chromosomes, his original observation was incorrect. Recently, Tjio and Levan (1956) and Tjio and Puck (1958) found 46 chromosomes for cells of this species in tissue cultures. Further, Kodani (1958) reported that 46, 47 and 48 chromosomes occurred in testicular material. Nakanishi (in press) has studied the chromosome number of cells from human fetal lung tissue. In primary culture he found the most frequent chromosome numbers to be 46 (25%), 47 (22%) and 48 (22%). These values closely approximated those obtained in the course of the present study. Painter (post-graduate seminar, University of Texas, 1958) has discussed Kodani's suggestion that it seemed certain that humans might show either 46, 47 or 48 chromosomes, the variation in number probably being due to the presence of a definite chromosome pair, a univalent, or the absence of an entire pair and pointed out that chromosome "fusion" was quite common in nature.

The newer techniques which have been applied for the observation of chromosomes in tissue cultures (cf., Hsu and Klatt, 1958; Nakanishi and Mizutani, 1959; Nakanishi, in press and in this paper) may be helpful in settling the vexatious uncertainty of the somatic number for man.

III. *Data on chromosomes in strain cultures.* Counts made from 32 cells of the Fernandes amnion strain fixed on the 35th day of incubation had a range from 60 to 77 with a peak at 67 (subtriploid number). Cells in the 40th subculture (195 days

from the date of the initial trypsinization) showed a range from 58 to 90 with a peak at 66. In the latest observations of 50 metaphase plates in the 84th subculture (664 days) the most frequent chromosome number was 75 (31 out of 50 cells) in a narrower range of 69 to 78. In these subcultures the chromosome counts apparently were in the triploid range and no diploid number was found (Figure 6). Examples of excellent metaphase plates which facilitated the examination of chromosomes are presented for reference (Figures 4, 5, 7, 8).

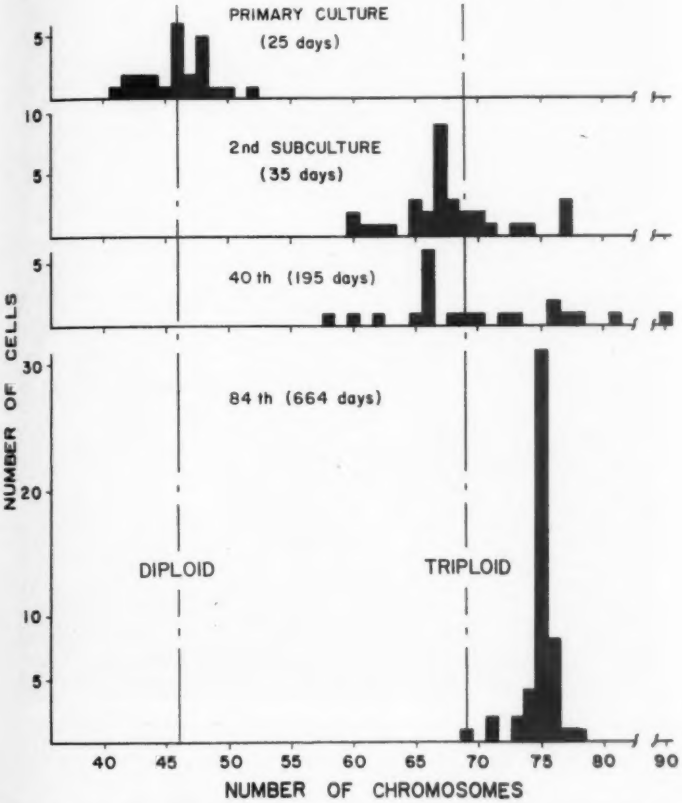
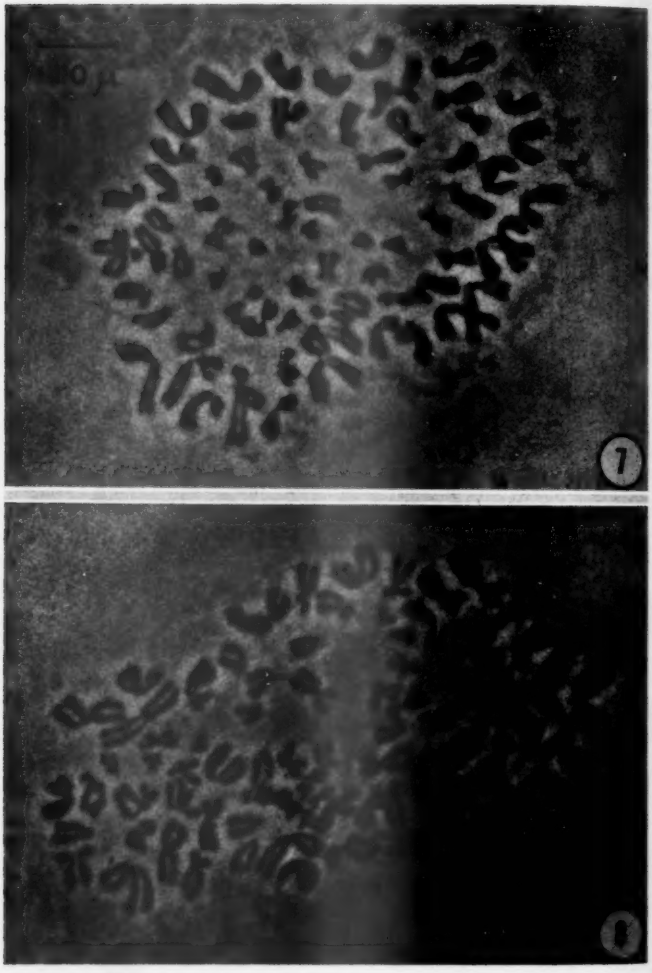


FIG. 6. Distribution of chromosome numbers in human amnion cells in primary and strain cultures.



FIGS. 7 and 8. Photomicrographs of chromosomes of the human amnion cell strain (75 chromosomes, respectively). FIG. 7 corresponds to figure 5. The magnification for figures 7 and 8 is given by the scale shown in figure 7.

As reported by many workers (cf. Levan, 1956; Hsu et al., 1957; Berman et al., 1957; Hsu and Klatt, 1959; Levan and Biesele, 1958; Nakanishi, in press) after continuous cultivation *in vitro*, cells of both normal and malignant origin exhibit extensive heteroploidy with very few or no euploids. Two human lung cell strains have been described (Nakanishi, et al.). The most frequent chromosome numbers were 76 and 77. In contrast, Syverton (1957) reported that in a strain of human tissue only diploids were found after many transfers. Recently, Tjio and Puck (1958) also stated that the chromosome number of cells from normal human skin, cervix and uterus remained constantly at 46 after more than 5 months of continuous, rapid growth in tissue culture involving scores of vessel transfers and a number of generations equivalent to many billions of progeny. Furthermore Ford and Yerganian (1958), in their studies on Chinese hamster cells, found that the diploids coexisted with the heteroploids for a long period.

Since the chromosome count made on the 35th day after the original trypsinization indicated a subtriploid number and also because the multiplicative rate of the cells was very high at this time, it was assumed that the strain was established before the 35th day (Fernandes, 1958). Levan and Biesele (1958) have reported that cells separated by trypsinization showed a higher incidence of chromosomal breaks than those which were mechanically minced. Further, they suggested that the reason trypsinization acted favorably in the establishment of new cell lines might be due to the appearance of cells with changed karyotype from the very beginning of tissue culture. The present observations would serve to supplement these data.

Summary

The chromosome numbers of human amnion cells were studied. The values obtained in freshly isolated elements before culture were 42, 48 and 55. The most frequent chromosome numbers in 25-day primary tissue culture preparations were 46 (25%) and 48 (21%). Counts made from cells of trypsinized cultures fixed on the 35th day of incubation (2nd subculture) had a range from 60 to 77 with a peak at 67. Cells in the 40th subculture (195 days from the date of the initial trypsinization) showed a range from 58 to 90 with a peak at 66. In the latest

observations in the 84th subculture (664 days) of the Fernandes amnion strain, the most frequent chromosome number was 75 (62%) in a narrower range of 69 to 78. The problem of determining the precise somatic chromosome number for man is discussed.

Acknowledgments

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REFERENCES AND NOTES

Notes accepted for publication, May 1959.

Berman, L., C. S. Stulberg and F. H. Ruddle: Human cell culture. Morphology of the Detroit strains. *Cancer Res.*, 17: 668–676, 1957.

Dunnebacke, T. H.: Cytopathic changes associated with poliomyelitis infections in human amnion cells. *Virology*, 2: 811–819, 1956.

Fernandes, M. V.: The development of a human amnion strain of cells. *Tex. Rept. Biol. and Med.*, 16: 48–58, 1958.

Fogh, J., and R. O. Lund: Continuous cultivation of epithelial cell strain (FL) from human amniotic membrane. *Proc. Soc. Exptl. Biol. Med.*, 94: 532–537, 1957.

Ford, D. K., and G. Yerganian: Observations on the chromosomes of Chinese hamster cells in tissue culture. *J. Nat. Cancer Inst.*, 21: 393–425, 1958.

Hsu, T. C.: Mammalian Chromosomes *In Vitro*. I. The karyotype of man. *J. Hered.*, 43: 167–172, 1952.

Hsu, T. C.: Numerical chromosomal variations in higher animals. In: *Developmental Cytology*, Ed. D. Rudnick, Ronald Press Co., New York, 1959.

Hsu, T. C., and O. Klatt: Mammalian Chromosomes *In Vitro*. IX. On genetic polymorphism in cell populations. *J. Nat. Cancer Inst.*, 21: 437–473, 1958.

Hsu, T. C., and O. Klatt: Mammalian Chromosomes *In Vitro*. X. Heteroploid transformation in neoplastic cells. *J. Nat. Cancer Inst.*, 22: 313–339, 1959.

Hsu, T. C., and C. M. Pomerat: Mammalian Chromosomes *In Vitro*. II. A method for spreading the chromosomes of cells in tissue culture. J. Hered., 44: 23–29, 1953.

Hsu, T. C., C. M. Pomerat and P. S. Moorhead: Mammalian Chromosomes *In Vitro*. VIII. Heteroploid transformation in the human cell strain Mayes. *J. Nat. Cancer Inst.*, 19: 867–873, 1957.

Kodani, M.: Thre chromosome numbers in Whites and Japanese. Science, 127: 1339–1340, 1958.

Levan, A.: Chromosome studies on some human tumors and tissues of normal origin, grown *in vivo* and *in vitro* at the Sloan-Kettering Institute. Cancer, 9: 648–663, 1956.

Levan, A., and J. J. Biesele: Role of chromosomes in cancerogenesis, as studied in serial tissue culture of mammalian cells. *Ann. New York Acad. Sci.*, 71: 1022–1053, 1958.

Makino, S., and T. Tanaka: Chromosome features in the regenerating rat liver following partial extirpation. *Tex. Rept. Biol. and Med.*, 11: 588–599, 1953.

Nakanishi, Y. H.: The development of a kitten lung cell strain and its chromosomes. *Zeit. f. Zellforsch.* (in press).

Nakanishi, Y. H., and M. Mizutani: Observations on the somatic chromosomes of lamb cells in culture. *Tex. Rept. Biol. and Med.*, 17: 134–141, 1959.

Nakanishi, Y. H., M. Mizutani and C. M. Pomerat: Smoke condensate on lung cells in tissue culture with special reference to chromosomal changes. *J. Nat. Cancer Inst.* (in press).

Oguma, K., and H. Kihara: Étude des chromosomes chez l'homme. *Archives de Biologie*, 33: 493–514, 1923.

Painter, T. S.: Studies in mammalian spermatogenesis. II. The spermatogenesis of man. *J. Morph.*, 37: 291–336, 1923.

Pomerat, C. M., M. V. Fernandes, Y. H. Nakanishi, and S. P. Kent: Irradiation of Cells in Tissue Culture. V. The effect of gamma irradiation from a cobalt^{60} source on human amnion cells *in vitro*. *Zeit f. Zellforsch.*, 48: 1–9, 1958.

Sachs, L., and M. C. Shelesnyak: The development and suppression of polyploidy in the developing and suppressed deciduoma in the rat. *J. Endocrin.*, 12: 146–151, 1955.

Syverton, J. T.: Comparative studies of normal and malignant human cells in continuous culture. *Spec. Publ. New York Acad. Sci.*, 5: 331–340, 1957.

Tanaka, T.: A study of the somatic chromosomes of rats. *Cytologia*, 18: 343–355, 1953.

Tjio, J. H., and A. Levan: The chromosome number of man. *Hereditas*, 42: 1–6, 1956.

Tjio, J. H., and T. T. Puck: Genetics of somatic mammalian cells. II. Chromosomal constitution of cells in tissue culture. *J. Exp. Med.*, 108: 259–268, 1958.

Walker, B. E.: Polyploidy and differentiation in the transitional epithelium of mouse urinary bladder. *Chromosoma*, 9: 105–118, 1958.

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THE APPLICATION OF MICROSPECTROPHOTOMETRIC TECHNIQUES TO WHOLE CELLS GROWN IN TISSUE CULTURE[a]

DONALD E. ROUNDS[b] AND C. M. POMERAT

Microspectrophotometry has been established as a valuable tool for histochemical analysis since the classical work of Caspersson (1936). Recent refinements of this technique have added the analysis of stained tissue sections in the visible range, which were considered to have a uniform thickness throughout the optical path area. It was recognized, however, that chromophores within a uniformly thick preparation are often of unequal distribution. Accuracy of histochemical estimations has been increased by either applying the two wavelength method of Patau (1952) or measuring crushed cell preparations, having physical characteristics which are advantageous for this technique (Davies, et al., 1954).

Whole epithelial type cells growing in a monolayer in tissue culture demonstrate a relatively two dimensional morphology which suggests itself as suitable material for analysis of this kind. Since reproducible histochemical techniques would greatly broaden the scope of tissue culture studies, an attempt was made to determine the validity of microspectrophotometric measurements as applied to isolated whole cells grown in tissue culture.

Materials and Methods

Tissue culture methods. The Fernandes strain of human amnion (Fernandes, 1958) was cultivated in Rose chambers for a period of 3 to 8 days in Eagle's medium with 10% horse serum. The Chang strain of conjunctiva and clonal HeLa S3 were grown on coverslips in roller tubes employing the same culture medium for a similar period of time.

Instrumentation. A Beckman model DU spectrophotometer, with the phototube housing removed, served as the constant voltage, monochromatic light source (Fig. 1). The light beam was reflected through the object on the stage of a Leitz microscope which was housed in a light-proof box. The image of the cell was further enlarged through a photomicrographic camera (Brinkman model 120 O) and was focused on its ground glass view plate (Fig. 2). During optical density readings, the mirror of the camera was displaced to allow the light beam to fall on the phototube of a Photovolt meter (model 520 M). The photosensitive cell was mounted on a film holder

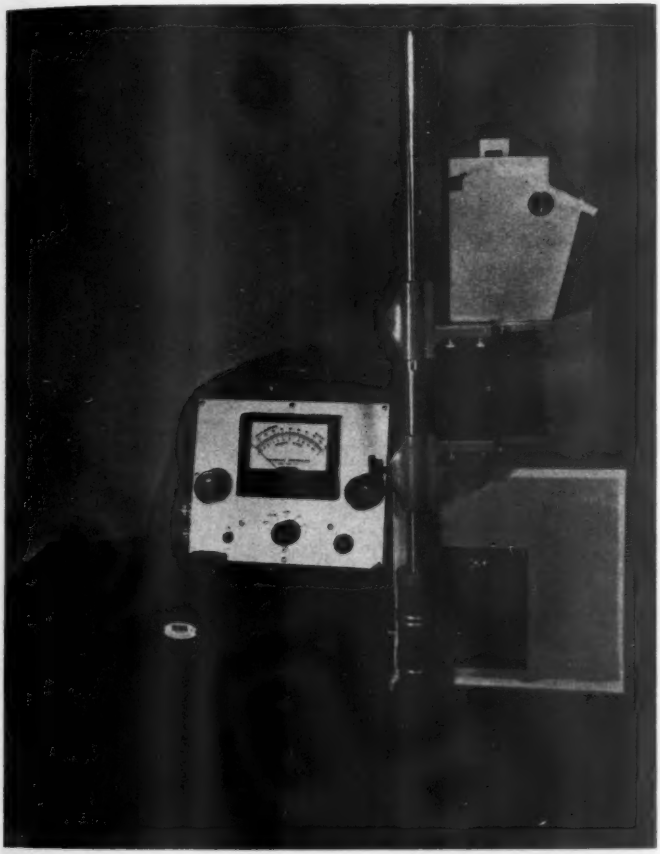

FIG. I. Setup for microspectrophotometry, with Beckman spectrophotometer, Leitz microscope, Brinkman photomicrograph, and Photovolt photometer.

frame at the top of the camera and had a circular exposed area of 3.14 cm^2. This represented an area of about 139 μ^2 at the level of the cell. With oil immersion optics, this area was sufficiently small to make measurements within regions of the nuclei or cytoplasm of single cells (Fig. 2). The high sensitivity of the photometer permitted full scale meter deflections with a slit jaw setting of about 0.1 mm, which insured the selection of a narrow region of the visible spectrum. With the aid of a mechanical microscope stage, images were placed in proper relationship to a ring on the ground glass view plate

which coincided in size and position with the exposed area of the photosensitive cell.

DNA determinations. Human amnion cells were maintained in Rose chambers for three to eight days before being fixed in neutral formalin. The cells were stained with the Feulgen reaction as described by Feulgen and

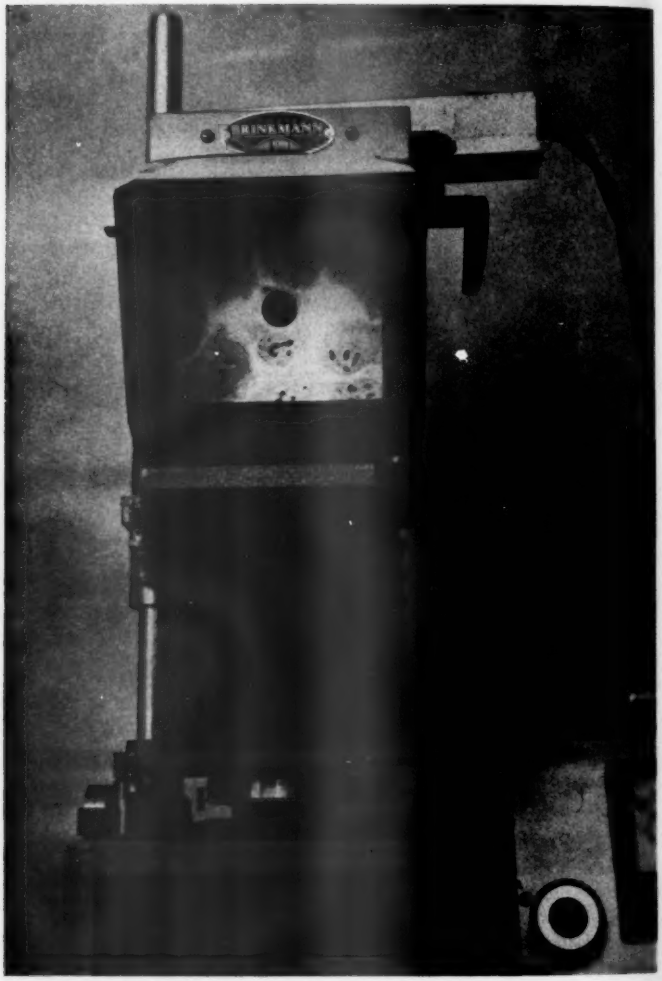

FIG. II. View plate showing an epithelial type cell in the optical path area.

Rossenbeck (1924), utilizing a hydrolysis time of 10 minutes. Optical density measurements were recorded for the fixed area of 3.14 cm^2 at a wave length of 546 $m\mu$. The area of the nucleus was traced from the image on the ground glass view plate of the camera and measured with a planimeter. Optical density of the total area was computed from the formula:

$$\text{Optical density of Total Area} = \frac{\text{Total nuclear area} \times \text{Optical Density of 3.14 cm}^2}{3.14\ \text{cm}^2}$$

Standard deviations from the mean were computed from the formula:

$$\sigma M = \sqrt{\frac{\Sigma(d^2)}{N(N-1)}}$$

Basophilia determinations. HeLa and conjunctival cells were cultured on coverslips in roller tubes in Gey's balanced salt solution for 2 to 12 hours prior to fixation. The cells showed no apparent damage while being maintained under these conditions. This technique reduced the number and size of pinocytic vacuoles and, as a result, increased the homogeneity of the cytoplasmic basophilia. Cultures were fixed in absolute alcohol and stained with May-Grünwald-Giemsa stains after the method of Jacobson and Webb (1952). The photometer was adjusted to full scale deflection, with no cell in the optical path, at a wave length of 525 $m\mu$. Optical density measurements were then recorded for the standard area in a representative portion of the cytoplasmic area. The area of the cytoplasm, minus the area of the nucleus, was traced from the view plate and measured with the planimeter. The optical density of the total cytoplasmic area was calculated as described above. Control slides were treated for two hours at 37° C in Gey's balanced salt solution containing 0.5 mg/ml ribonuclease at a pH of 6.8.

Determination of the absorption maxima. The maximum absorption of the Feulgen reaction has been reported by Feulgen and Rossenbeck (1924) to be 546 $m\mu$ and confirmed many times in recent years. The May-Grünwald stock solution (3 mg per 100 ml absolute alcohol) was diluted with absolute alcohol to 1:50 and 1:100 dilutions. The absorption curves of these dilutions were determined in a Beckman spectrophotometer with alcohol serving as the blank. The absorption curves for similar Giemsa stain dilutions (stock solution = 8 mg per 50 ml alcohol and 50 ml glycerin) were also determined, but with distilled water serving as the solute and the blank. The absorption curve for stained cytoplasm was determined as described for basophilia measurements, except that wave lengths throughout the visible spectrum were scanned.

Results

Approximately 24 amnion interface nuclei for each of 4 cultures were measured for the total amount of Feulgen positive chromophores. The optical density measurements for the total nuclear area are listed in Table 1. For purposes of comparison, data taken from comparable measurements (Pollister and Ornstein, 1955) of 6 μ sections of rat liver parenchyma cells are given

TABLE 1

Optical Density Measurements of Nuclei Stained with the Feulgen Reaction

Amnion Strain Nuclei		Rat Liver Parenchyma Nuclei*	
Cell Nos.	Mean E_{546}	Cell Nos.	Mean E_{546}
1–21	0.198±.015	1–25	0.136±.008
22–45	0.183±.013	26–52	0.144±.008
46–70	0.202±.013	53–79	0.138±.010
71–95	0.202±.010		
Summary	0.197±.002	Summary	0.140±.005

* Data taken from Pollister and Ornstein (1955).

in the same table. Summarized data on 79 nuclei, reported by Pollister and Ornstein, gave standard deviations of about 3.5%. The simple one wave length method of measurement applied to 95 nuclei of whole cells gave standard deviations from the mean of about 1%. Higher mean values for DNA in amnion nuclei reflect the polyploidy of this cell strain, described by Fernandes (1958).

Figure III indicates the absorption curves for May-Grünwald stain in solution and cytoplasmic basophilia stained with Jacobson's method. The May-Grünwald stain shows an absorption peak at 525 mμ for two concentrations of dye, as does stained basophilia. Figure IV illustrates that the absorption maximum for two concentrations of Giemsa stain was 520 mμ. Both May-Grünwald and Giemsa stains in solution demonstrate a linear relationship of dye concentration with optical density, as can be seen in figure V. Thus, each of the stains in solution satisfies the conditions of Beer's law (E=kc) within a range of dye concentration comparable to the density of stained basophilia. Table 2 lists optical density measurements of 50 HeLa and conjunctival cells stained with Jacobson's method. The small standard devia-

TABLE 2

Optical Density Measurements of Cytoplasmic Basophilia Stained with Jacobson's Method

Cell Type	No. Cells	Mean E_{525}	Per Cent Dev.
Conjunctiva	50	1.649±.060	3.63
HeLa	50	1.245±.047	3.75

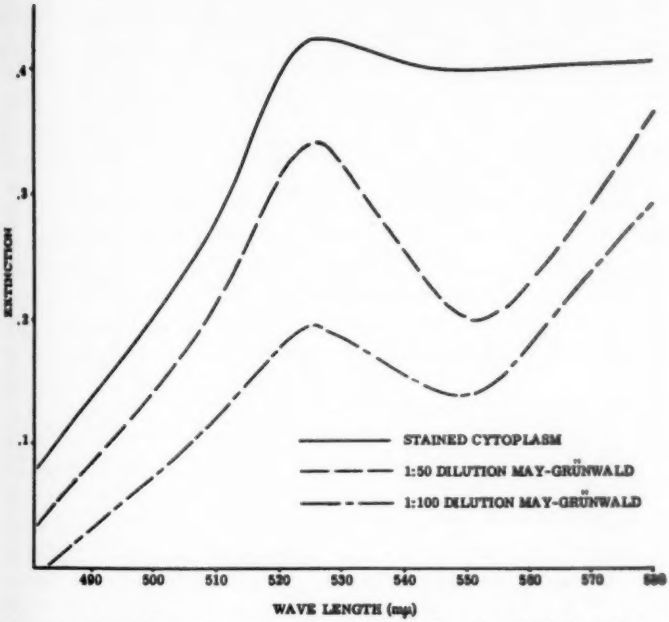

FIG. III. Absorption spectrum of May-Grünwald stain solutions and the spectrum of a cell stained with Jacobson's method.

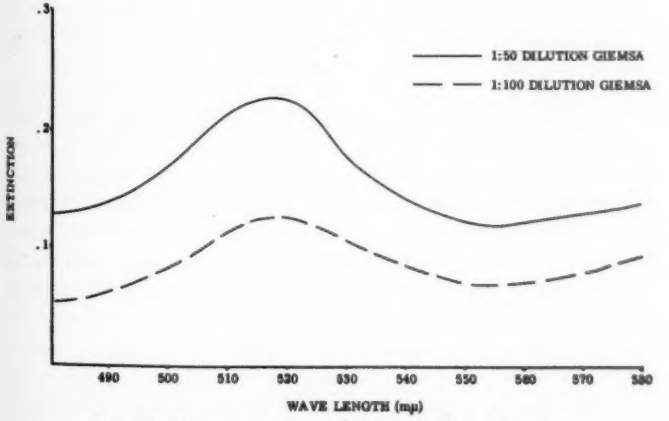

FIG. IV. Absorption spectrum of Giemsa stain solutions.

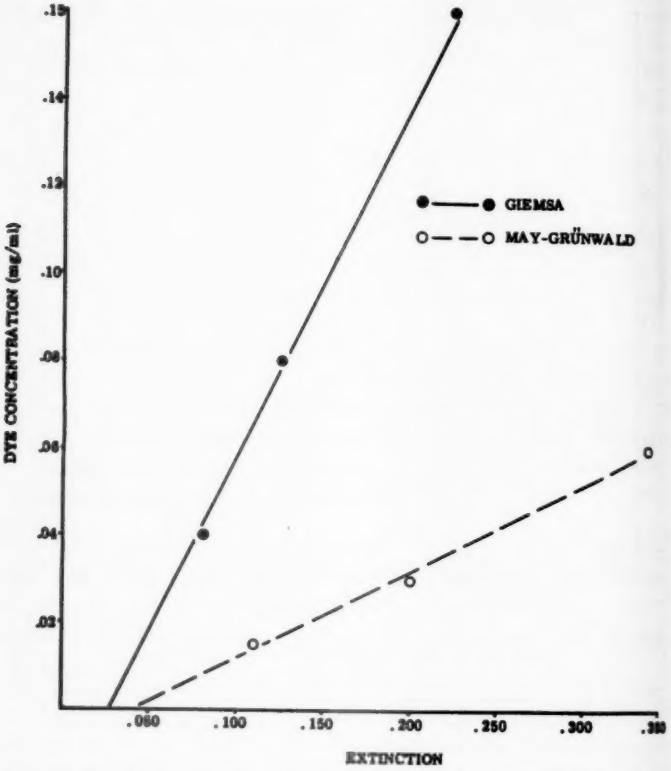

FIG. V. Standard curves for Giemsa and May-Grünwald stains, showing a linear relationship between dye concentration and optical density

tion from the mean (less than 4%) suggests a reproducible stoichiometric relationship between stain and basophilia. Ribonuclease treated control cultures revealed cells with no measurable basophilia. This confirmed the report of Jacobson and Webb (1952) that the blue coloration of the cytoplasm represents ribonucleic acid or ribonucleoprotein.

Discussion

A high degree of specificity of the Feulgen reaction for DNA has been demonstrated by a number of workers (Review by

Swift, 1955) and a comparison of cytological measurements with direct analysis by Ris and Mirsky (1949) has indicated an adherence of this reaction to Beer's law. Interface nuclei from many cell strains in tissue culture show a relatively uniform distribution of chromatin material when stained with the Feulgen reaction. Those cells which had a maximum surface area on the glass cover slip contained flattened, spheroid nuclei with physical properties approaching those of the crushed cell preparations described by Davies, et al., (1954). Estimations of the Feulgen positive material have been successfully applied to cultured rat (Bloch, 1953) and chick (Firket, et al., 1955) fibroblasts. Analyses of the Feulgen positive chromophores in epithelial type elements, in this work, are primarily reported as a check on the type of instrumentation used.

Jacobson and Webb (1952) have demonstrated that the use of the May-Grünwald-Giemsa staining method results in a reproducible differential stain with a specificity for the two nucleoproteins similar to the Feulgen and methyl green-pyronin techniques. Michaelis and Granick (1945) have reported that most metachromatic stains in solution deviate from Beer's law by forming new absorption peaks with variations in the dye concentration. The data in figures III and IV give no evidence that the May-Grünwald-Giemsa stains fall into this category within the range of dye concentrations used for measurement. The linear relationship of the concentration of each stain in solution with optical density of the solution indicates a reasonable conformance with Beer's law (figure V).

One major difference between cells in tissue culture with sectioned material was that the distance of the optical path through the whole cells was not controlled. Variations in thickness throughout the living cell were likely to occur, as well as variations in the mean thickness from cell to cell. These variations were reduced to a considerable extent as a result of cell shrinkage during alcohol fixation. Because the cells adhered to the glass, the total area remained constant, but the reduction in cell volume was expressed by a flattening of the cell. Since extinction varies in direct proportion with the distance through the absorbing layer ($E=kd$), reproducibility of extinction measurements suggested that whole cells were of a relatively uniform thickness after fixation.

Davies, et al., (1954) have stated that errors in microspectrophotometry are due to (1) parts of the object being out of focus, (2) glare, (3) image deformation due to diffraction of small parts of the object, and (4) light scatter. A further source of error in measurement of the basophilia concentration, was the inability to consider the chromophore content above and below the nucleus. The data in Table II, showing standard deviations from the mean of less than 4%, suggest that these sources of error remained at a minimum level.

A number of advantages became apparent with the application of this method of measurement to whole cells. First, optical density measurements are more likely to consider the total chromophore content of the cell. Since some portion of a sectioned cell can not be considered during optical density measurements, it is assumed that the neglected portion contains an unknown concentration of chromophores which should contribute to the total amount. Secondly, cells which demonstrate a highly differentiated morphology often show a heterogeneous distribution of chromophores. If those cells are allowed to disperse from an explant in tissue culture, the chromophores are likely to be found in a more random, homogeneous distribution. In addition, by controlling the culture medium, homogeneity may be further increased by reducing pinocytic vacuolization. As a result, the less arduous one wave length method of measurement can be applied with reasonably consistent results. Thirdly, most cells which adhere to a cover slip in tissue culture assume a relatively two dimensional morphology with a maximum surface area. This characteristic often permits the area of the optical path to fall entirely within a representative area of the cell. Fourthly, monolayers of cells, in areas where populations are somewhat sparse, contain easily detectable peripheral margins. Areas of measurement in tissue sections, which include cells of dense population, could be contaminated with portions of two or more superimposed cells. Finally, cells in tissue culture can be fixed, stained, and mounted on the coverslips on which they grew, without requiring the additional procedure of embedding and sectioning.

Although conformity to Beer-Lambert relationships have been discussed as a necessary control for this method of analysis, reproducibility was also dependent upon adherence to established

criteria for selection of the cells to be measured. Those criteria included selection of cells (1) having an area large enough to accommodate the area of the optical path, (2) showing a relatively homogeneous distribution of chromophores, and (3) having distinct peripheral margins. For measurement of cells growing from primary explants, it would be necessary to select areas which were free of debris, fibers, or the plasma clot. By applying these criteria to epithelial type cells, it was concluded that the monolayer of cells in tissue culture assumes physical characteristics which lend themselves to a simple, reliable application of microspectrophotometry.

Summary

Microspectrophotometric techniques were utilized to estimate the chromatin and basophilia content of epithelial type cells grown in tissue culture. The validity of measurements on whole cells as compared with more universal measurements on cell sections was discussed. It was concluded that the physical characteristics of whole cells in tissue culture rendered them uniquely suited for this type of histochemical analysis.

REFERENCES AND NOTES

Bloch, D. P.: Effect of Colchicine on Synthesis of Desoxyribonucleic Acid in Tissue Cultured Rat Fibroblasts. *Proc. Soc. Exper. Biol. and Med.* 84: 341–346 (1953).

Caspersson, T.: Uber den chemischen Aufbau der Strukturen des Zellkernes. *Skand. Arch. Physiol.* 73: Suppl. 8, 1–158 (1936).

Davies, H. G., M. H. F. Wilkins, and R. G. H. B. Boddy: Cell crushing: a technique for greatly reducing error in microspectrometry. *Exptl. Cell Res.* 6: 550–553 (1954).

Fernandes, M. V.: The development of a human amnion strain of cells. *Tex. Rep. Biol. and Med.* 16: 48–58 (1958).

Feulgen, R. and H. Rossenbeck.: Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und darauf beruhende elektive Färbung vom Zellkernen in mikroskopischen Präparaten. *Z. phys. Chem.* 135: 203–248 (1924).

Firket, H., S. Chévremont—Comhaire, and M. Chévremont: Action of ribonuclease on living cells *in vitro* and synthesis of desoxyribonucleic acid. *Nature* 176: 1075–1076 (1955).

Jacobson, W. and M. Webb: The two types of nucleoproteins during mitosis. *Exptl. Cell Res.* 3: 163–183 (1952).

Michaelis, L. and S. Granick: Metachromasy of basic dyestuffs. *J. Amer. Chem. Soc.*, 67: 1212–1219 (1945).

Patau, K.: Absorption microphotometry of irregular-shaped objects. *Chromosoma* 5: 341–362 (1952).

Pollister, A. W. and L. Ornstein, in "Analytic Cytology" (R. C. Mellors, ed.), McGraw-Hill, New York, (1955).

Ris, H. and A. E. Mirsky: Quantitative cytochemical determinations of desoxyribonucleic acid with the Feulgen nucleal reaction. *J. Gen. Physiol.* 33: 125–146 (1949).

Swift, H., in "Nucleic Acids" Vol. II, (E. Chargaff and J. N. Davidson, eds.) Academic Press, New York, (1955).

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FOUR-PI RADIOCHROMATOGRAPHY PROTOTYPE OF A DETECTOR

LOTHAR L. SALOMON[a]

In scanning radiochromatograms of low-energy beta emitters, difficulties are occasionally encountered due to absorption of radiation, chiefly within the paper itself and in the window of the detector. The problem of absorption by windows has been successfully solved by employing windowless or ultrathin window detectors. Another possible approach is here described, which makes use of the fact that radiation is emitted in approximately equal amounts both above and below the chromatogram. By scanning the radioactive spots simultaneously above and below the chromatogram with low-background detectors connected in parallel to a single input of the ratemeter, it has been possible to obtain a substantial increase in sensitivity. Thus, using the tandem detectors described below with 1 mg./cm.^{2} aluminized windows, approximately 50% of the sensitivity to C^{14} of a windowless single detector operating under closely similar conditions was obtained. This means, in effect, that similar tandem systems, with ultrathin windows of the order of 100 γ/cm.^{2}, or windowless, should be particularly useful when spots of low activity, questionable significance or of very weak beta emitters are being scanned.

Structural details of a prototype of the upper of the tandem detectors are shown in figures I and II. The lower detector is identical except that holes for the bolts holding the detectors together are threaded. The body of the detector is made of Lucite plastic, using a block approximately $1\frac{1}{2}\times3\frac{1}{2}\times4$ inches. Dimensions indicated in figure I are suitable when strips $1\frac{1}{2}$ in. wide are being scanned. The hole for the detector is most advantageously located as close to the paper as possible, but no dimension is indicated except as "minimum" distance since the care of machining the block, or nature of the material if other than Lucite, will be the determining factors. Cathodes and anodes are built in as shown in the lower drawing of figure II. A and B are neoprene rubber stoppers provided with glass tubes for gas, and for anode and cathode leads. The leads are 18 ga.

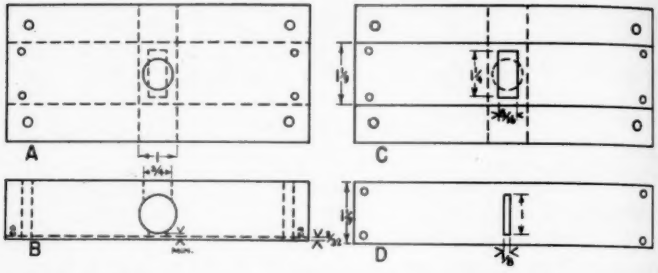

FIG. I. A, B, C, top, side, and bottom views, respectively, of upper of the two Lucite blocks. D, brass plate with 1 x 1/8 in. slit. Head-on view of hole for detector, shown in B. The detector should be minimal distance away from groove for paper strip and brass plate. Please note that the width of brass plate D and the corresponding slot in the Lucite block is erroneously given as 1 1/9 in., but should be 1 9/16 inch.

(B. and S.) copper wire, flattened at one end. Dark ridges in the glass tubing are thickened portions, intended to center the leads. The cathode lead is soldered to cathode C, which is a thin sheet (0.002 in. thick in this case) of 2-9/16×1-20/32 in. copper with rounded corners. The cathode is prepared by brief etching with dilute HCl (1 volume conc. acid plus 5 volumes of water), rinsing with water and blotting dry. The anode is 0.003 in. tungsten or stainless steel wire (for example 40 ga. stainless steel wire suture). It is connected to the leads by folding the flattened portion of the lead, inserting the end of the anode, and making a firm union by flattening with pliers and soldering. Glass tubes for leads are sealed with Apiezon W wax, or other suitable material. Windows are affixed with plastic cement, such as Du Pont Duco. The choice of material for windows may be dictated by special requirements. In the present model, 0.00025 in. polyester film, Opaque A, aluminized on both sides, total thickness 1 mg./cm.^{2} (Alexander Vacuum Research, Inc., New York, N.Y.) is being used. Finally, the brass plate D is affixed with recessed screws. Gas connections are shown in figure II.

The chromatogram is transported by taping one end to the paper of the recorder chart, or by a separate device. The former procedure with the recti/riter, Model RR1M-A16T (Texas Instruments, Inc., Houston, Texas), which is provided with a gear shift for 10 speeds, is illustrated in the upper drawing of figure II. Because the effective diameter of the uptake spool is always

slightly greater for the chromatogram than for the chart, there is a small but linear deviation between the chart record and length of chromatogram with this method. The deviation with a chromatogram of Whatman No. 1 paper, 2 ft. long, was found to be between 3 to 5 mm. overall, so that the difference between location of radioactive spots and peaks on the chart was quite small. However, radioactive reference spots may be employed to eliminate chance of error. Since the effective diameter for uptake of the strip is greater than that for the uptake of the chart

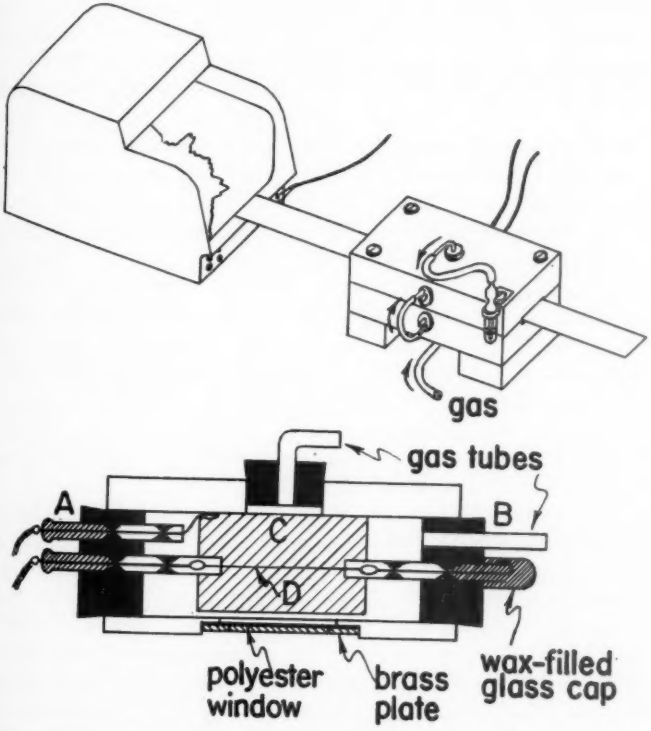

FIG. II. Upper drawing, apparatus assembled, showing path of quenching gas. Lower drawing, cross section through detector. External ends of glass tubing closed with sealing compound, except for gas tubes. The copper wire holding anode D is bent over the side of the glass tube in stopper B, to permit stretching, and is insulated with a wax-filled glass cap.

by a constant amount, the deviation will be no greater for each of a number of radiochromatograms of equal length, spliced together, than it will be for a single one.

The instrument exhibits some photosensitivity, which is not unusual. This is effectively counteracted by shielding against light with a coat of dense black paint on the exterior surfaces, or by covering with black cloth. Performance characteristics of the tandem detectors now in use are as follows when used in conjunction with a Tracerlab, Inc. (Waltham, Mass.) Model P-11 preamplifier (which is not required) and helium, saturated with absolute ethanol at 0° C. Starting potential, about 1225 volts; plateau length, more than 350 volts; slope of plateau, maximum approximately 8% per 100 volts between 1250 and 1400 volts, less than 0.5% per 100 volts between 1400 and 1650 volts; background, unshielded except against light, about 50 c.p.m.; background, shielded with 2 in. of lead, less than 30 c.p.m.; sensitivity to beta radiation emitted by C^{14} in comparison to the Scanogram Model RSC-5 (Atomic Accessories Inc., Bellerose, N.Y.) windowless chromatogram scanner, using commercial quenching gas for the latter, at equal chart speed and time constant, about 50%. Ten sections cut from a chromatogram equal in width to the slit (⅛ in.) were compared in the Tracerlab Model SC-16 windowless flow counter. In this case, somewhat higher relative sensitivity, about 65%, appears to exist. However, the range was from 47 to 91% indicating that this value, which is an average, may be in error and too high. The variation encountered is probably the result of difficulties in locating the exact spot counted in the tandem system.

The use of windows results in economy in consumption of quenching gas, and eliminates problems arising from entry of abraded paper articles or of vapors into the tubes. Although satisfactory performance for many purposes can be gained from an instrument essentially as described, improvements embodying the principle of tandem detectors may be made. The ultrathin window has been mentioned. In a more sophisticated model now in construction, the neoprene stoppers are replaced by threaded Teflon plugs, and the body of the detector is made of metal which, by serving as the cathode, does away with the need for copper sheets and associated leads.

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INDIVIDUAL VARIABILITY WITH REFERENCE TO CEREBROSPINAL FLUID AMINOACID PATTERNS*

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Among the various biological fluids, aminoacid composition of Cerebrospinal fluid (C.S.F.) has received little attention. Perhaps it could be attributed to the want of easy availability of the material and minuteness in quantity of the aminoacids in C.S.F. The few reports available on this topic have been reviewed by several authors (4, 5, 8).

The present paper reports observations made during studies on aminoacid profiles of CSF from mental patients.

Materials and Methods

The material used in this study comprised of CSF samples obtained from ten male schizophrenic patients (from the chronic ward of the Mental Hospital, Bangalore). They were inpatients for more than two years and their ages ranged from 25–40 years. The samples of CSF were obtained at three periods separated by monthly intervals. The patients were receiving no treatment during the period of study.

The samples obtained were subjected to analysis of routine components (viz. cells, chlorides, glucose, proteins as also Lange's Colloidal test and W. R) using standard techniques (3).

Unidimensional paper chromatography was used to study the aminoacid composition. Hydrolysis and desalting of the samples were done using the technique of Baliga *et al.* (1). The color development, identification and quantitative estimation employed the technique of Giri *et al.* (2). The details of the chromatographic technique have been discussed in a previous publication (5). The chromatographic technique was checked using standard aminoacids (Roche) treated in the same manner. Agreeing values were checked from duplicate samples. Besides this the total of all the aminoacids in hydrolysed samples follows closely the total protein content of the original fluid.

Observations

The routine analysis of the fluids did not reveal deviations from the normal range at any time.

The values obtained for the various aminoacids showed that the range of variability was very high in the group of patients Further, the aminoacid composition of the fluids studied during the three stages revealed that despite low variability in any one

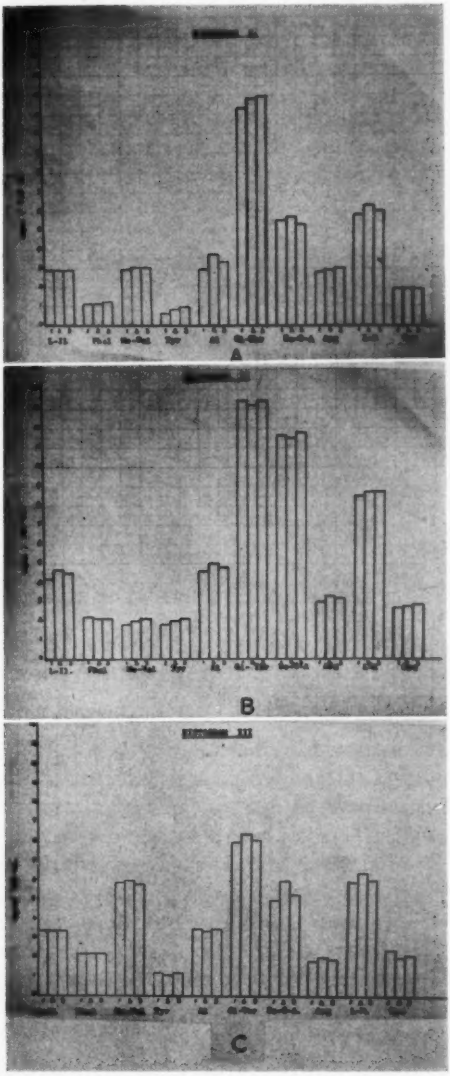

FIG. I. Histograms of three individuals (A-B-C) showing the low variability of their amino acid patterns at three monthly intervals. Note the relative constancy of the patterns.

individual with respect to any one aminoacid, there was marked difference from individual to individual. Since the values of the three stages for an individual patient varies within narrow limits the average value for the three stages for the various aminoacids in the group has been plotted on the histogram. It was observed that with reference to CSF aminoacid profiles there exists an intraindividual constancy during the three stages of the experiment, but with interindividual differences throughout.

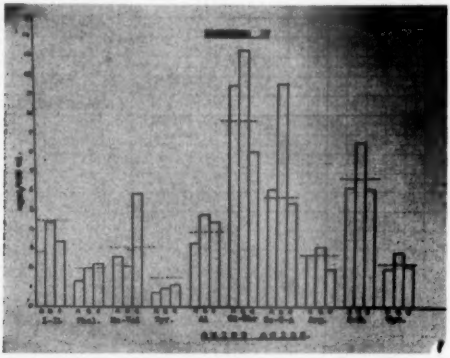

FIG. II. A histogram of the average CSF amino acid patterns for three individuals compared. The dotted line is the average for a group of ten individuals.

Discussion

That each individual has a biochemical make up which may be relatively constant throughout's one's span of life and is little influenced under normal conditions by external factors is becoming increasingly clear. This has been stressed by Williams (9) in his recent book. Similar observations have been made in animals and men with reference to aminoacid composition of blood and urine, and diet has been found to have little influence (10). The present findings with reference to CSF aminoacid patterns also appear to parallel the above noted observations. However, this has to be considered in the light of possible influence of: aminoacids of external (through diet) or internal (through blood) source, influence of physiological factors and influence of psychiatric status and comparison with a set of controls. As regards the influence of aminoacids of extraneous origin all the patients were solely on

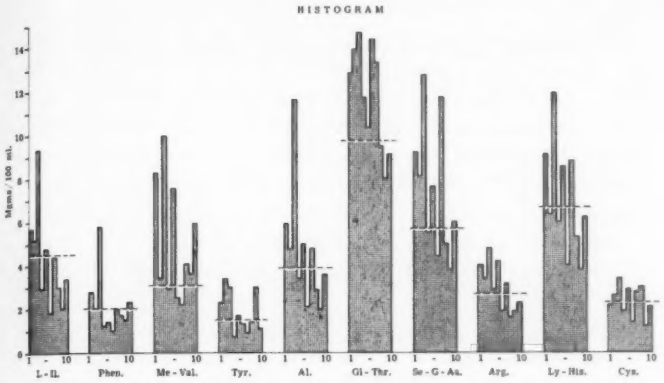

FIG. III. A histogram comparing the amino-acid patterns in the CSF of ten individuals. Each column combines three determinations taken at monthly intervals. The dotted line is the average of the group of ten. L.IL is leucine-isoleucine; Ph.al is phenyl alanine; Me.val. is methionine-valine; Tyr. is tyrosine; Al. is Alanine; Gl. Thr. is Glutamic acid, threonine; St. Gly. Aa. is serine, glycine, aspartic acid; Arg. is arginine; Ly. His. is Histidine; Cys. is cystine.

identical hospital diet for more than two years and diet has no immediate influence over acinoacid composition of CSF; very few aminoacids can traverse the blood brain barrier (6) and it has been shown that no relationship exists between aminoacids of blood and CSF (7). No pathophysiological changes have been observed in the brain of schizophrenics (6). No psychiatric episodes occurred in the patients of the group at any time during the period of study. This study could not be conducted on controls but since the patients were studied at three stages, each serves as one's own control.

The significance of such variability observed in a fluid located in such a specialized organ as the central nervous system, however, needs to be revealed.

Summary

The aminoacid composition of Cerebrospinal Fluid in a group of schizophrenic patients has been reported. The inter-individual differences observed among the patients have been discussed in the light of the general phenomenon of biochemical individuality.

Acknowledgments

The author is grateful to Professor Roger J. Williams (Director, Clayton Foundation Biochemical Institute, University of Texas) for his kind gesture in communicating the paper; to Dr. M. V. Govindaswamy (Director, All India Institute of Mental Health, Bangalore) for his constant guidance and encouragement; to Dr. C. V. Natarajan and Dr. S. Balakrishnan for helpful suggestions. The author is indebted to the Ministry of Education (Government of India) for the award of a Research Training Scholarship and to the Clinical and Nursing Staff for their co-operation.

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REFERENCES AND NOTES

1. Baliga, B. R., *et al.*: "A simple method for desalting biological fluids for chromatography," *J. Ind. Inst. Sci.*, 34: 18–22, 1955.
2. Giri, K. V., *et al.*: "Circular Paper chromatography—Part VI—Quantitative determination of aminoacids," *J. Ind. Inst. Sci.*, 35: 145–180, 1953.
3. Gradwohl, R. B. H., *Clinical Laboratory Methods and Diagnosis*, (Vol. II), C. V. Mosby & Co., St. Louis, 1957.
4. Kemali, D., *et al.*: "Ulteriori richerche ed indiagni comparative sugli aminoacidi liberi del liquido cefalo-rachidiano umano," *Acta. neurol.*, 12: 419–433, 1957.
5. Rao, Sridhara Rama, B. S., and Govindaswamy, M. V.: "Studies on the Cerebrospinal fluid," *J.A.I.I.M.H.*, 1: 47–59, 1958.
6. Richter, D.: "Biochemical aspects of Schizophrenia" in *Schizophrenia, Somatic aspects*, Pergamon Press, London (1957).
7. Solomon, J. O., *et al.*: "Free aminoacids in CSF," *J. Biol. Chem.*, 171: 695, (1947).
8. Walker, B. S., *et al.*: "Aminoacids of the Cerebrospinal Fluid," *A.M.A. Arch. Neurol. and Psychiat.*, 73: 149–158, 1955.
9. Williams, R. J.: *Biochemical Individuality*, John Wiley & Sons, Inc., N.Y., 1956.
10. Williams, R. J.: *The University of Texas Publication, No. 5109* (1951).

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RADIOAUTOGRAPHIC OBSERVATIONS ON REGENERATION OF TRANSITIONAL EPITHELIUM

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Transitional epithelium in the mouse urinary bladder consists of three cell layers, each with its own specific type of cell (10). In other stratified epithelia, cells are produced in the basal layer and then move to the surface while differentiating (4, 12). However, in transitional epithelium of the mouse, the histogenesis of surface cells indicates that they develop independently of the deeper cell layers (10).

The main feature of this independent development is the conversion of diploid surface cell nuclei to the polyploid state in the fetal mouse bladder. The polyploid surface cells so produced then divide to make more polyploid surface cells, and the other cell layers apparently do not contribute to the further growth of the surface layer. The various phenomena of surface cell histogenesis led to the hypothesis that a differentiation of nuclei to the polyploid state was essential for the completion of cytoplasmic differentiation (10). One test of this hypothesis would be to damage transitional epithelium in the adult mouse bladder and observe the process of regeneration. If polyploid nuclei were essential to the formation of surface cells, then new cells would have to come either from other surface cells that had survived damage or from basal cells which would pass through a phase of polyploidization. If polyploid nuclei were not essential to surface cell differentiation, then basal cells might produce surface cells directly, without undergoing any nuclear change. The intermediate cell layer would be a potential complicating factor in such a test since at least some of its nuclei are tetraploid.

Labeling the deoxyribonucleic acid (DNA) of dividing cells with a radioactive nucleic acid precursor, and then following these labeled cells with radioautography, is an ideal method for studying the origin and migration of newly formed cells (12). The most specific precursor of DNA is thymidine, and when combined with tritium it can produce radioautographs of high

resolution (3, 8), because tritium emits beta particles of low energy which produce ionizations within the emulsion close to the source of radioactivity.

The following report deals with the regeneration of transitional epithelium in the mouse urinary bladder after mechanical injury. The origin and fate of newly formed cells, labeled with thymidine-H^3, is determined by radioautography.

Materials and Methods

The lower abdominal wall of heterozygous male mice was cut open under ether anaesthesia, and the urinary bladder was punctured with a pointed scalpel blade. The inner surface of the bladder around the puncture was scraped with the scalpel point, and the abdominal wall was sutured. This treatment produced a burst of mitoses in the epithelium 24 hours after operation. To label these dividing cells, 100 μc of thymidine-H^3 was injected subcutaneously 18 hrs. after operation. Animals were sacrificed at 1 day after injury (6 hrs. after the injection of isotope) and at 2, 3, 4, 5, 8 and 16 days after injury. Tissues were fixed in Carnoy's (Bouin's fixative gave inferior results). The bladders were sectioned and prepared for radioautography. Radioautographs were made by the method of Messier and Leblond (6) with a few modifications. Since this procedure has been used for more than 1000 radioautographs in this laboratory with fairly consistent results, it will be described here in detail.

Paraffin sections are mounted on albuminized slides labeled with glass marking ink. After drying, the albumin-glycerol adhesive is also spread over the back of the slides. Paraffin is removed in xylol and the slides are run through absolute alcohol; absolute alcohol-ether, 1:1; and then left 15 minutes in a 1% solution of celloidin (6) in alcohol-ether. The slides are dried 15 minutes, given another quick dip in the celloidin solution, and then dried overnight. Subbing fluid (2) consisting of a 0.5% solution of gelatin containing 0.05% chrome alum (left overnight in an oven at 37° C to dissolve the gelatin), is used as an emulsion binder in which the slides are dipped and then allowd to dry for about 2 hrs. The coating of slides with emulsion is performed in a darkrom at 60° F. Although low humidity has been recommended (6), the humidity in our darkroom has been as high as 80%, without any deleterious effects noticed. A Kodak Wratten Series 2 safelight is used. Liquid NTB3 emulsion (Eastman Kodak, Rochester) is placed in a Naples staining jar (Thomas, Philadelphia) and melted in a water bath at 42–44° C for 1 hour. The slides are placed on a warming plate at the same temperature for a few minutes, then dipped slowly into the emulsion and held there about 4 seconds. After withdrawing the slide, its base is held against absorbent paper briefly and then the slide is put on a plastic rack. Slides should be checked occasionally for uniform coating by holding them up two feet in front of the safelight. The emulsion coating should be relatively even, free of bubbles, and opaque. Emulsion is left on the back of the slide to diminish the tendency for peeling during later dehydration procedures.

The drying of slides after dipping in emulsion (or after subbing fluid) is accomplished by standing them on plastic racks at a slight tilt from the vertical position. A simple rack holding 20 slides can be constructed from ⅛″ (inch) acrylic plastic sheets. A strip 4″ x 27″ serves as a base. A second strip 2½″ x 27″ is cemented along the middle of the base at a 110° angle, being braced by small strips of plastic at the back (acute angled) side. A third plastic strip ½″ x 27″ is cemented to the base in front (the obtuse angled side) of the second strip, with a ½″ gap left between them. The top ¼ of the radioautographic slide will then rest against the second strip and the bottom edge of the slide will be braced between the third strip and the base. With two of these racks, 40 slides can be coated with emulsion in about 1 hr. When the racks are filled, the safelight is turned out and the slides left to dry for 4 to 6 hrs. The slides are then filed in Bakelite boxes, 10–15 slides per box, with the remaining space occupied by a small (1¾″ x ⅞″ x ¾″) plastic box (Althor, Brooklyn) having holes drilled (or burned) in the lid and containing indicator Drierite. The boxes are then sealed with masking tape. These precautions for keeping slides dry during exposure were shown by Messier and Leblond (6) to be essential. The slide boxes are stored upright in the refrigerator at 6° C. With the dosage used in this experiment, slides can be developed at periods from 1 to 6 months. Emulsion in the Naples jar can be returned to the refrigerator and reused 3–6 times during a 4-month period; more extended usage tends to result in too much background fog.

Development of radioautographs can be carried out in D76 at 68° F for 3 miuutes or in fine grain developers for longer periods (usually 15 minutes). The slides are rinsed briefly in water and cleared for 8 minutes in acid fixer. Subsequent processing is carried on at room temperature in the light and consists of: running water, 5 min.; Harris hematoxylin, acid varient (5), 3 min.; water rinse; acid bath (2 cc. conc. HCl in 300 cc. H_2O), 2 sec.; running water, 5 min.; dehydration in graded alcohol series, including eosin Y counterstaining; 15 mins. in 2 changes of absolute alcohol; 1 hr. in alcohol-cedarwood oil, 1:1; 1 hr. in dilute (⅔ toluene) Permount; 1 hr. in stock Permount; allow slide to drain 5 sec., dip cover glass in dilute Permount and place on slide; clean off excess mounting medium after slides have dried. Staining intensity is somewhat variable with this method (compare Figs. 2 and 3). Alternative staining procedures have been described by other authors (1, 7). The foregoing specifications are not critical, but sometimes slight changes in radioautographic procedure can lead to unsatisfactory results. It is advisable to concentrate on one procedure until good radioautographs are obtained and then continue to use that method without variation.

Results

Radioautographs of urinary bladder taken 24 hrs. after injury (6 hrs. after injection of thymidine-H^3) show many foci of silver deposition in the emulsion over transitional epithelium, but no reactions over muscle and very few over connective tissue. Therefore, cell proliferation in response to injury is limited to the epi-

thelium at this time. Since all the experimental animals were injected at the same time, and since thymidine-H^3 probably remains available to cells for less than 2 hrs. (3), it is obvious why transitional epithelium was the only tissue that could be followed radioautographically in this study. A few radioactive nuclei did appear in the connective tissue and muscle at later time intervals, but they belonged to migrating leucocytes and not to proliferating cells.

Radioactive nuclei were rare in the transitional epithelium at the site of puncture (P, Fig. 1). Starting at some distance from the puncture, masses of radioactive nuclei were found (arrows, Fig. 1), which extended along the epithelium for varying distances, being interspersed with areas having few or no reactions. All three cell layers contained radioactive nuclei (Fig. 2), with many of the reactions occurring over the basal and intermediate

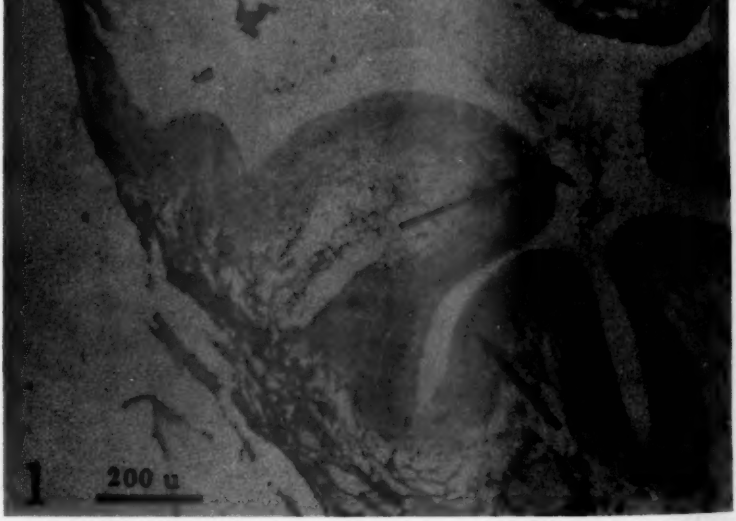

FIG. I. Radioautograph of urinary bladder 24 hrs. after pncturing with a scalpel (at P) and 6 hrs. after injection of thymidine-H^3. Radioactive cells are absent from the transitional epithelium around the site of puncture, but become increasingly frequent further away (arrows).

layers and fewer over the surface layer. However, the ratio of radioactive to non-radioactive nuclei in a given layer was similar in all three layers, since there are many more deep cells than surface cells in the epithelium. Radioactivity in one cell layer was usually accompanied by radioactivity in the adjoining layers. Occasionally, radioactive nuclei could be found in the surface layer without any radioactivity being present in the adjoining deeper layers in the same section (Fig. 2) or in adjacent sections.

The distribution of radioactive nuclei was equivalent to the distribution of mitotic figures at 24 hrs. after injury, although the number of radioactive nuclei was much greater than the number of mitoses, as would be expected. The aggregations of silver grains over surface cell nuclei were, on the average, considerably larger than those over basal cell nuclei (Fig. 4), and thus were proportional to the larger size of these surface cell nuclei. Also,

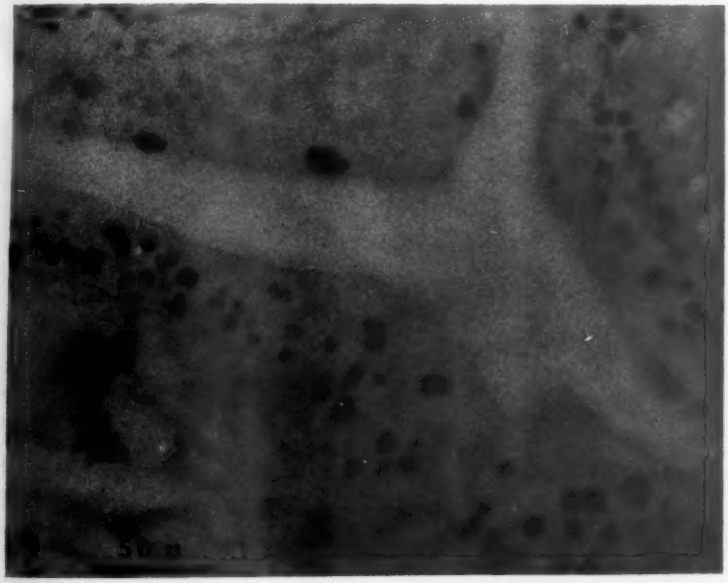

FIG. II. Radioautograph of transitional epithelium 24 hrs. after injury. Radioactive nuclei are abundant throughout most of the epithelium, but only surface cell nuclei are radioactive in the strip of epithelium at the upper left.

mitotic figures in surface cells were larger than those in basal cells.

In bladders taken 2, 3, 4, 5, 8 and 16 days after injury, the regenerating transitional epithelium produced radioautographic patterns essentially the same as in the 1 day specimens (compare Figs. 2 and 9). Theoretically, the cells should have divided at least once; therefore, the radioactive nuclei should have been in pairs after the first day. The radioautographs could be interpreted in this way, but since the radioactive nuclei tended to occur in large groups, even at the 24 hr. period, the distribution of reactions was not favorable for the identification of pairing (Figs. 2 and 3). In some areas, studied in serial sections at 3, 8 and 16 days after injury, groups of radioactive surface nuclei were found without any radioactive deep cell nuclei nearby. These clusters were probably products of surface cell divisions. However, there was no significant generalized decrease with

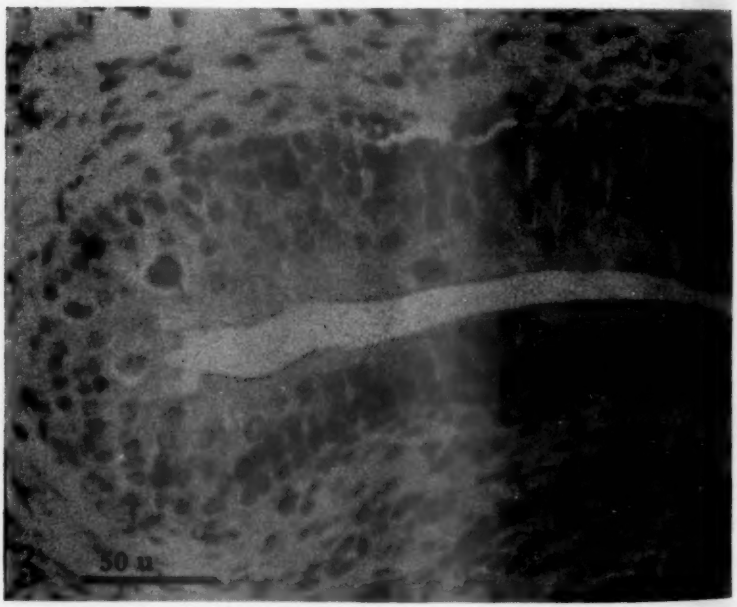

FIG. III. Transitional epithelium 16 days after injury. Notice the similarity of the radioautographic pattern to that in FIG. II.

time in the density of silver grain deposition per nucleus, so the nuclei could not have divided many times. Some mitotic figures could still be seen in the epithelium at 2, 3, and 4 days after injury, although they were much less frequent than at 1 day. Mitoses were rare after 4 days.

There was no significant shift in the distribution of radioactive nuclei between cell layers, and at 16 days radioactive nuclei were still present in all cell layers without any sequence of reactions to indicate that nuclei in one layer were giving rise to nuclei in another (Fig. 4). The total number of radioactive cells varied somewhat at the different time intervals, perhaps due to variation in the severity of injury caused by the scalpel blade. However, the similarities in the radioautographic patterns at all 7 time intervals were very great and the differences only minor.

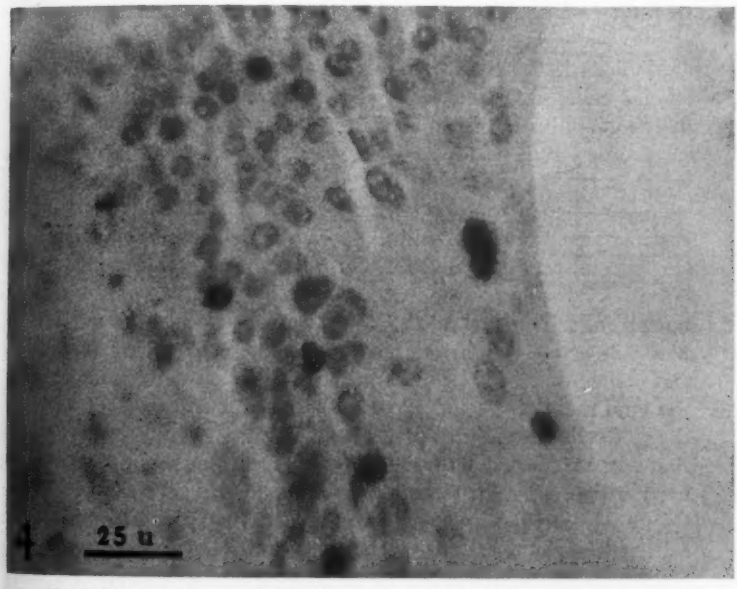

FIG. IV. A small area of epithelium 16 days after injury. There is no evidence of cell migration, or diminution of radioactivity per nucleus. The size of the aggregation of silver grains over the surface cell nucleus in the center of the photo is greater than that over any of the nuclei in the deep layers. The radioautographs illustrated in FIGS. I to IV were stained with hematoxylin and eosin.

Discussion

The large, polyploid (10) nuclei in surface cells of transitional epithelium are capable of undergoing mitosis in the adult mouse. This is evident from the large mitotic figures that appeared in the surface layer at 24 hrs. after injury and is further supported by the widespread incorporation of thymidine-H^3 (indicating DNA synthesis) in surface cell nuclei. The greatest number of radioautographic reactions was over nuclei in the layer of basal cells, the second greatest number was over the intermediate cells, and the least number was over the surface cells. This is in direct proportion to the population size of each cell type and is precisely the distribution of DNA synthesis that would be expected if each cell population were replacing only cells of its own type.

The radioautographic pattern in regenerating transitional epithelium is very different from that of a stratified squamous epithelium. In a study of cell proliferation and migration during the estrus cycle (9), it was shown that the basal cells of the vaginal epithelium incorporated thymidine-H^3 and then produced new labeled cells that migrated toward the lumen. The result was a marked diminution in their content of radioactivity, but at the same time a continuous trail of radioactive nuclei was produced, which extended from the basal layer to the superficial layers. No such sequence of proliferation and migration was seen in regenerating transitional epithelium during the 16 day period studied. On the contrary, the distribution of radioactive cells remained essentially constant over this period. Therefore, it appears that no cell migration took place, aside from the initial migration of non-radioactive cells over the wound, and that the loss of cells was compensated by a generalized proliferation which increased the total mass of the epithelium without any differential migrations of cells laterally or toward the lumen. It can be concluded that when surface cells are lost they are replaced mainly, or perhaps entirely, by the division of other surface cells (of course, this conclusion applies to a situation where many healthy surface cells are present to act as a source of new cells).

These experiments were designed to test the hypothesis that the conversion to polyploidy during the development of surface cells is an essential step in their differentiation. Although the results reported here do not prove this theory, they do provide additional support for it. Considering the fact that surface cells are

highly differentiated (11) and their nuclei are polyploid (10) it is surprising that they divide so readily and replace their own type instead of being replaced by the basal cells which show no differentiation with the electron microscope (11) and have normal diploid nuclei (10). This replacement of cells by the division of differentiated surface cells, instead of by undifferentiated basal cells as would occur in most stratified epithelia, is evidence in favor of the hypothesis that surface cells require polyploid nuclei, especially since polyploid nuclei are readily produced by divisions of nuclei that are already polyploid, whereas it would require special circumstances, such as prevail in the embryo bladder (10), for polyploid nuclei to be produced from nuclei that are only diploid.

Summary

Injury of transitional epithelium with a scalpel resulted in widespread mitotic activity 24 hours later. Using thymidine-H^3 as a label, the fate of these cells was studied radioautographically. There was no evidence of cell migration over a 16 day period (except for the movement of non-radioactive cells to cover the wound). Apparently, cell loss was compensated by a general increase in epithelial mass without any differential migration laterally or toward the lumen. Thus, during regeneration, surface cells were replaced mostly or entirely by division of other surface cells. These results are consistent with the theory that polyploidy is essential for the differentiation of surface cells.

REFERENCES AND NOTES

1. Bergeron, J A.: Controlled staining of autoradiographs. *Stain Tech.*, 33: 221–223, 1958.
2. Fitzgerald, P. J.: *Analytical Cytology;* Ed., R. D. Mellors: Blakiston, New York, 1955.
3. Hughes, W. L., V. P. Bond, G. Brecher, E. P. Cronkite, R. B. Painter, H. Quastler, and F. G. Sherman: Cellular proliferation in the mouse revealed by autoradiography with tritiated thymidine. *Proc. Nat. Acad. Sci.*, 44: 476–483, 1958.
4. Leblond, C. P. and Bruce E. Walker: Renewal of cell populations. *Physiol. Rev.*, 36: 255–276, 1956.
5. Lillie, R. D.: *Histopathologic Technique and Practical Histochemistry.* Blakiston, New York, 1954.
6. Messier, B., and C. P. Leblond: Preparation of coated radioautographs by dipping sections in fluid emulsion. *Proc. Soc. Exp. Biol. Med.*, 96: 7–10, 1957.

7. Olivereau, Madeline: Influence de quelques colorants en histoautoradiographie. *Bull Micros. Appliq.*, 6: 100–105, 1956.
8. Taylor, J. H., P. S. Woods, and W. L. Hughes: The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. *Proc. Nat. Acad. Sci.* 43: 122–128, 1957.
9. Walker, Bruce E.: Cell production and migration in the female mouse genital tract. *Anat. Rec.*, 133: 459, 1959.
10. Walker, Bruce E.: Polyploidy and differentiation in the transitional epithelium of mouse urinary bladder. Chromosoma, 9: 105–118, 1958.
11. Walker, Bruce E.: A comparative study of transitional epithelium with the electron microscope. Anat. Rec., 130: 385, 1958.
12. Walker, Bruce E., and C. P. Leblond: Sites of nucleic acid synthesis in the mouse visualized by radioautography after administration of C^{14} labeled adenine and thymidine. *Exptl. Cell Research*, 14: 510–531, 1958.

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A NEW MICROINJECTION SYSTEM USING STEEL PERFUSION CHAMBERS[1]

SAUL WISCHNITZER*

Many physiologists, tissue culture workers, and cytologists are interested in the microscopic study of the growth and metabolism of living cells. These investigators are especially anxious to determine the reactivity of cells and their components to different chemical environments and the identification of the various chemical products of cellular activity. To carry out such investigations an apparatus is required which permits the withdrawal and replacement of samples of the medium for assay, or the quantitative introduction of chemical reagents without disturbing the preparations. In addition, observation of the preparations at all levels of magnification is necessary. In recent years, a number of systems which take into consideration the above mentioned factors have been reported, (Buchsbaum and Kuntz 1954, Schwöbel 1954, Von Stosch 1954, Worst 1954). It is the purpose of this paper to describe a newly designed chamber which can be used both for short term and continuous maintenance and a microinjection unit for short term use that can be assembled from inexpensive commercially available equipment.

Microscope

The microinjection equipment is arranged to operate in association with an inverted microscope (Fig. 1). The advantages of such an instrument has recently been discussed in detail (Wischnitzer 1958). The microscope shown in Figure 1 was patterned after that originally constructed by Leitz for R. Chambers (1950). An increasing number of inverted microscopes are now commercially available.

Microinjection Unit

The microinjection unit itself consists of a hand "volumette"[2] mounted on a base. This unit is placed on a support platform which is attached at an appropriate height to a ring stand. A

rubber pad was placed between the support platform and the volumette base to avoid sliding.

A 1/10 ml. Mohr pipette graduated into hundreds, was modified so as to fit into the rubber diaphragm of the volumette while a standard ground glass Luer tip was adapted to the delivery end of the pipette (Fig. 2). Attached to the Luer tip was a Luer slip

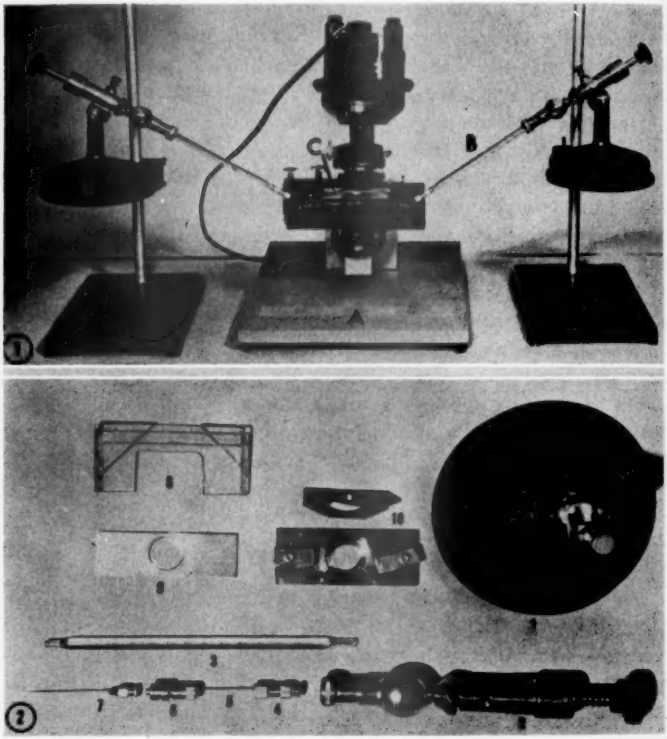

FIG. 1. Microinjection apparatus; consisting of an inverted microscope (A), the volumette and its accessories (B), and the injection chamber unit (C).

FIG. 2. Component parts of FIG. 1.

(B) Volumette:
1. Base
2. Hand volumette
3. Graduated 1/10 ml. Mohr. pipette.,
4. Luer-slip adaptor
5. Polyethylene tubing-PE-50
6. Luer lock adaptor
7. 2" hypodermic needle # 24

(C) Chamber:
8. Lucite holder for chamber
9. One-piece injection chamber
10. Two-piece injection chamber

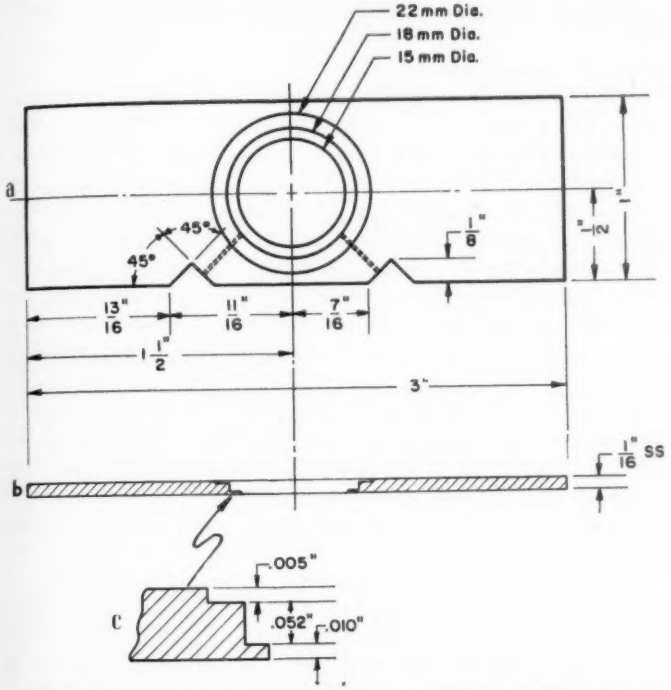

FIG. 3. A diagrammatic representation of a one-piece perfusion chamber illustrating its appearance and dimensions when seen from above (a) in sagittal section (b) and an enlargement of the chamber portion of the slide in sagittal section (c).

adaptor which was connected to a Luer-lock adaptor by fine (PE 50) polyethylene tubing. A 2 inch No. 24 Luer hypodermic needle attached to the Luer-lock adaptor,[3] was inserted into the chamber.

Chamber

Numerous chambers particularly those for use in tissue culture work have been described (see reviews of earlier literature Rose 1954, Dick 1955, Toy and Bardawil 1958). The most essential criteria in the construction of chambers, especially when phase contrast optics are to be used, is that the surface be parallel, have a uniform thickness and not be separated from each other by

more than 1 mm (more if long working distance optics are used). Two stainless steel chambers, both conforming to these requirements, are presented.

The first chamber (Fig. 3) has the advantage of simplicity in construction since it is made up of one piece (not counting two cover slips which are always part of every chamber). The chamber measures $3'' \times 1'' \times 1/16''$ and has a hole in its center of three different widths as a result of its step-like margin. The upper and lower levels are for the cover slips whereas the middle level, namely the area delimited by the coverslips and the walls of the slide, is the chamber proper. (The circular coverslips are kept in place by the use of melted beeswax). Two holes, each being $0.022''$ in diameter for inserting the hypodermic needles, are drilled at a 45° angle between the upper and lower step.

The second chamber (Fig. 4) is made up only of two pieces and in reality is almost identical with the one previously described except that modifications are made to secure the top coverslip and thereby the entire chamber. This is done by adding a top piece (Fig. 2) which is simply a plate having a hole in its center around which projects downward a short ($0.15''$) rim which fits into the upper level of the chamber and presses down on the margins of the coverslip. This top piece is kept in place by two metal hinges which are attached by screws to the slide itself and can be tightened when the chamber is in use. In addition, the margin of the upper step and chamber proper where the needle passes, is in part notched out and fitted with a small square rubber "gasket" which permits the bore of the hole of the remaining metal portion to be larger. These modifications facilitate movement of the needles and allows arranging the set-up with greater ease. In addition, the beneficial feature of withdrawing the needles with the chamber still remaining sealed, is permitted.

After each use, either chamber can be easily sterilized. A simple lucite holder (Fig. 2) can be made to fit the slide chamber permitting use of a mechanical stage.

Summary

A microinjection system is described which can be easily assembled for short term biochemical and pharmacological investigations of cells and their components. In addition, two steel

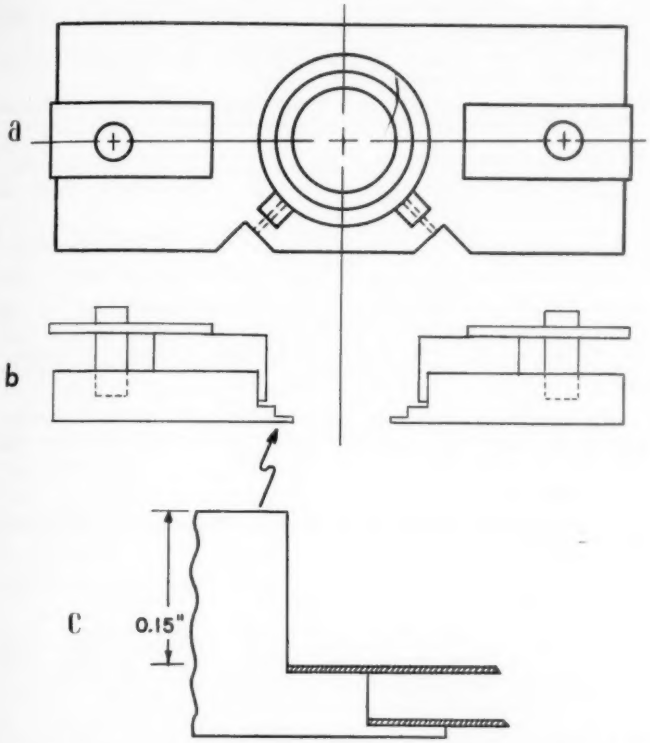

Fig. 4. Diagrammatic representation of a two-piece perfusion chamber in the same positions as shown in FIG. 3. The critical dimensions are the same as for the one-piece chamber, except for the allowance made for the projecting rim of the top piece which helps keep the upper coverslip fixed (c). In the sagittal section (b), the top piece held down by the metal hinges is seen.

chambers of one or two piece construction are presented which can be used in continuous perfusion experiments.

REFERENCES AND NOTES

Buchsbaum, R. and J. A. Kuntz: The effects of certain stimulants and depressants on individual fibroblasts in a perfusion chamber. *Ann. N.Y. Acad. Sci.*, 58: 1303–1310, 1954.

Chambers, R. and M. Kopac: in McClnng's *Handbook of Microscopic Technique.*, 492. P. B. Hoeber, Inc., 1950.

Dick, D. A. T.: An easily made tissue culture perfusion chamber. *Quart. J. Micr. Sci.*, 96: 363–369, 1955.

Rose, G.: A separable and multipurpose tissue culture chamber. *Tex. Rep. Biol and Med.*, 12: 1074–1083, 1954.

Schwöbel, W.: Eine kammer zur mikroskopischen untersuchung von Zellsuspensionen. *Mikroskopie*, 9: 302–305, 1954.

Von Stosch, H. A.: Eine kammer für mikroskopische Lebenduntersuchungen mit Trenung von Objekt und strömendem Medium. *Protoplasma*, 44: 365–368, 1954.

Toy, B. L. and W. A. Bardawil: A simple plastic perfusion chamber for continuous maintenance and cinematography of tissue cultures. *Exptl. Cell Research*, 14: 97–103, 1958.

Wischnitzer, S.: The Inverted Microscope: its history and use as a cytological tool. *Mikroskopie*, 13: 39–44, 1958.

Worst, J.: A Microinjection system. *Quart. J. Micro Sci.*, 95: 469–472, 1954.

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[1] This work was performed in part under the auspices of the U.S. Atomic Energy Commission while the author was Postdoctoral Resident Research Associate at the Argonne National Laboratory.

[2] Wilkens-Anderson Co., Chicago, Illinois.

[3] All Luer connections and related items are obtainable from Clay-Adams, Inc., New York.

LAURENCE-MOON-BARDET-BIEDL SYNDROME*

Review of the Literature and a Report of Five Cases Including a Family Group With Three Affected Males

JOHANNA BLUMEL AND WILLIAM T. KNIKER[a]

Eugenic advice was sought by the parents (Chart 1) of a child exhibiting features of the Laurence-Moon-Bardet-Biedl (LMBB) syndrome, composed of the following six cardinal features: obesity, oligophrenia, genital dystrophy, polydactylism, retinitis pigmentosa, and familial occurrence. The possibility of having another affected child was of the utmost importance to this couple. Although the classical picture composed of all the above features is relatively rare, innumerable cases of the so-called incomplete forms of this syndrome have been reported. These range from cases with one or more of the signs missing to those with one or more being replaced by concomitant anomalies listed by various authors and increasingly reported with every additional publication on this condition.

FIG. 1. Photograph of proband, Case one (Chart 1-IV-5) taken at age 16 weeks.

Our interest concerns the etiological aspect of the LMBB syndrome, which from a genetic point of view remains enigmatic. Although the hereditary importance has been recognized and a number of excellent studies reported in the literature (11, 27, 35, 44 and 47), there is as yet no unanimity of thought among the professions regarding the etiology of this condition. One of the major problems confronting the investigator involves the question of what actually constitutes the syndrome, and the inclusion or exclusion of the so-called doubtful or incomplete cases represents a real problem. Thus an attempt was made to bring the literature up to date, re-examine and if possible elucidate the factors responsible for this condition.

Thorough reviews and summaries of the literature regarding the clinical, pathological and etiological features of the LMBB syndrome since it was first described in 1866 by Laurence and Moon (26), and later associated with additional abnormalities by Bardet (2) and Biedl (4) in the early nineteen twenties, have been published by Reilly and Lisser (37); Sorsby, Avery and Cockayne (43); Warkany, Frauenberger and Mitchell (47); Bisland (6); Cockayne *et al* (10); Rolleston (39); Burn (9); Brattgard (8); Ornsteen (34), and Macklin (27). In the present report we are adding five cases of this syndrome, three in one family, and reviewing the literature.

Case Reports and Pedigrees

The pedigrees presented diagramatically in Charts 1, 2 and 3, represent several generations. The Roman figures on the left indicate generations, and the Arabic figures locate individuals. These pedigrees were established by examination of medical records, correspondence with persons who attended the people, and personal interviews with the parents of the proband in Chart 1, with the proband herself in Chart 2, and the mother of the proband in Chart 3.

CASE ONE (33846-M) Chart 1, IV-5:

Following a full term uncomplicated pregnancy, this white male was spontaneously delivered on September 5, 1956, weighing 4 lbs. 15 oz. Neonatally, there were frequent episodes of "strangling with mucus" and facial and acral cyanosis. On the fifth day an episode of cyanosis lasting several minutes occurred associated with rolling upward of the eyes, apnea, stiffening of the body; this was followed by three hours of deep sleep. On

that day a heart murmur was discovered; because of a fever of 103 F (R) Oxytetracycline was prescribed for two weeks. Since then circumeral and nasal cyanosis occurred daily during handling or with crying; otherwise, the infant was in no particular distress. A slow feeder, the infant took three to four ounces of an evaporated milk formula every three to four hours.

A large "soft" area over the top of the head had been present since birth. The left iris was abnormally shaped; the patient was said to see "light" and hear well. With exertion "hard" breathing occurred. The penis was congenitally grown to the scrotum, the urethral opening on the ventral surface of the penis at the scrotum. Buds representing sixth fingers and toes were clipped off bilaterally on the first day of life.

Referred to the Children's Hospital at the University of Texas Medical Branch for evaluation of the multiple congenital (especially cardiac) defects, the patient was admitted on October 29, 1956, then seven weeks old. Examination revealed that the temperature, pulse, and respirations were within normal limits. Weight was 6 lbs. 3½ oz., (10 percentile for birth), length was 49 cm. (10 percentile for birth), and head circumference was 37.5 cm. (97 percentile for birth). The infant was well-developed, slender, irritable, in no apparent distress. Circumoral cyanosis occurred with crying. The normally proportioned head seemed relatively large, and on palpation, a "ping-pong ball" sensation was present over the neurocranium at the vertex involving the parietal and occipital bones. Although scalp veins were prominent, no fontanelle enlargement or bulging was noted. The pupils reacted to light weakly; a coloboma involving the inferior portion of the left

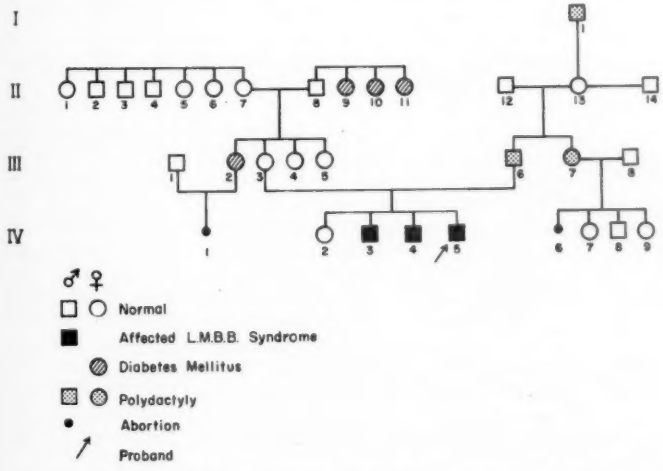


CHART 1. A pedigree showing three affected brothers in one family. Cases 1, 2 and 3.

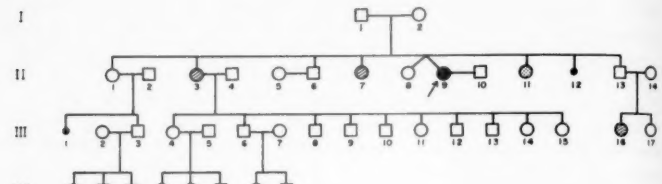


CHART 2. A pedigree showing Case 4.

iris was present. There was bilateral nystagmus and external strabismus of the left eye. The heart was not enlarged to the left, but cardiac dullness extended three centimeters to the right of the sternum in the fourth interspace. There was normal rhythm and rate was 120 per minute. A prominent rough systolic murmur, heard in all cardiac areas, was loudest in the third interspace to the left of the sternum. A third degree hypospadius was present, the urethral meatus on the ventral surface of the flexed penis at the junction of the corona with the scrotum. The testes were descended. Minimal syndactyly between the second and third toes bilaterally was seen. The infant was responsive with good Moro and sucking reflexes. The deep tendon reflexes were generally hyper-reactive.

During the hospital stay, hemoglobin levels varied between 11.2 and 15.5 gm. %; there was usually a moderate leucocytosis with normal differential count. Urine analyses were negative until mid-December; subsequently albuminuria, microscopic hematuria, pyuria and casts were common. Serum, calcium, phosphorus, alkaline phosphatase, and electrolytes were normal. The blood non-protein nitrogen was slightly elevated until December when it became normal. An electroencephalogram revealed impaired activity over the left hemisphere. Although the first electrocardiogram showed left ventricular hypertrophy, several later studies demonstrated marked right axis deviation and right ventricular hypertrophy. Cardiac fluoroscopic examination revealed right heart enlargement, diminished pulmonary vascularization and small hilar shadows. Angiocardiograms demonstrated a septal (ventricular) defect and possible pulmonary hypertension. Skull films and long bone survey were negative. A cystogram was negative; retrograde pyelograms showed tortuosity of both ureters with slight enlargement of calyces in the right renal pelvis.

On the second hospital day a several-minute episode of cyanosis, limpness, tachycardia of 200 occurred; after a brief period of cardiac asystole, the

infant completely recovered. A very slow feeder, the infant took an evaporated milk formula until early December when it was switched to Lonalac, (R) due to development of edema. Psychometric examination at ten weeks of age revealed a motor function level of four weeks indicating severe deficiency. Opthalmologic consultants noted bilateral estropia and no retinitis pigmentosa. In December, cystoscopic examination demonstrated bladder trabeculation and vesicle neck contracture; an indwelling catheter was left in place. The patient was discharged December 27, 1956, on Lonalac(R) infant soft diet, Oxtetracycline prophylaxis, indwelling urethral catheter and multivitamin drops. The patient fed poorly at home and had intermittent diarrhea and abnormal distention related to oral administration of various kinds of antibiotics. Because the catheter became plugged and would not stay in place, the patient was readmitted on February 22, 1957.

At that time temperature was 100 F (R), pulse 160, and respirations 40. Length was 56 cm., weight 8 lbs. 14 oz., and head circumference 41 cm. The patient was poorly nourished and appeared chronically ill. The anterior fontanelle was open, measuring four by four cm., the posterior fontanelle was not as yet closed, and all sutures were slightly separated. Head could not be supported on neck. Deep tendon reflexes were absent. No edema was seen. The remainder of the examination was as before.

Laboratory studies were essentially unchanged. Microscopic red blood cells and white blood cells remained in the urine with occasional albuminuria. NPN was 22 mg. %. Urine culture grew out *E. coli* and paracolon bacilli. Skull and chest roentgenograms and cardiograms had not changed.

The patient was placed on Novobiocin orally and remained afebrile. On February 26, 1957, gavage feedings were instituted because of anorexia. On

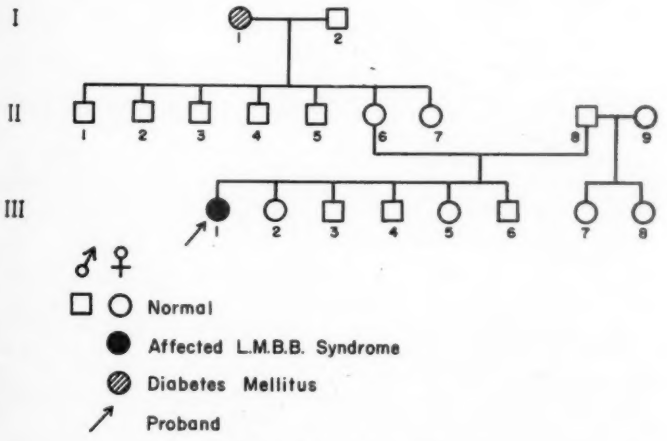


CHART 3. A pedigree showing Case 5.

February 28 irritability and abdominal distention appeared; bowel sounds were active; gastric suction relieved the distention immediately. Early the next day these signs recurred associated with higher pitched bowel sounds. At 8:00 a.m. the infant was found dead in bed.

Post mortem examination

Pathological Findings. (Dr. Brooks, Dept. of Pathology)

Gross findings: Development and nutrition appeared poor; length was 56 cm. (Less than 3 percentile for 3 months); weight was 8 lbs. and 14 oz. (75–90 percentile for birth). The head appeared relatively too large. An inferior coloboma was present in the left iris. The abdomen was markedly distended. There was a third degree hypospadius; both testes were located in the scrotum. A small umbilical hernia was evident.

Serous sacs: Normal, without excess fluid.

Respiratory system: Entirely normal.

Cardiovascular system: The right ventricle was enlarged and dilated; its wall was hypertrophied. The pulmonary artery was ⅓ the diameter of the aorta. There was no recognizable pulmonary valve, but a pinhole opening was present at the origin of the pulmonary artery. A 5 mm. defect was located high in the interventricular septum.

Alimentary system: A diverticulum of the ileum occurred in its terminal portions. The entire small intestine and ascending colon were dilated, hemorrhagic, and purple up to a point 18 cm. from the cecum. Here the intestines passed under a loop of small bowel; beyond this point the colon and rectum were normal. The ascending colon was located on a mesentery. Other abdominal organs were normal.

Genito-Urinary system: Both kidneys were of normal size except that the right renal pelvis was dilated, filled with purulent material and exhibiting hydronephrosis. Bladder, ureters and testes appeared normal.

Endocrines: Thyroid, adrenals, pituitary, and thymus glands appeared normal. The parathyroid glands could not be found.

Lymphatic system: There were many small firm, reddish nodes in the mesentery.

Skeletal system: Except for "ping-pong ball" sensations on pressing the top of the skull, there were no abnormalities.

Brain: The brain weighed 797 gms. and exhibited very small, torturous gyri on sagittal section, the entire corpus callosum was absent except in the region of the anterior part of the genu. Moderate hydrocephalus, greatest in the occipital region, was obvious. The aqueduct of Sylvius was patent.

Microscopic:

Lungs: There were focal areas of atelectasis alternating with other areas of distended alveoli, containing mononuclear cells of abundant, pale cytoplasm, probably macrophages. The histology suggests a lipoid pneumonia, secondary to aspiration sometime before death. Fat stain with oil Red O demonstrated only a few fat droplets in the phagocytic cells.

G. I. tract: In the region of malrotated bowel involved in volvulus, cell outlines were smudged and appeared to be undergoing early infarction.

Bladder and prostate: There was acute inflammation of the urinary bladder. An abscess was noted between the prostate and seminal vesicles.

Brain: The architecture of the gyri was normal, but there were many pyknotic nuclei, and evidence of edema and vascular congestion. The aqueduct was patent. There were partial absence of ependyma of the ventricular system, apparently secondary to hydrocephalus, and moderate gliosis adjacent to the enlarged ventricles.

CASE TWO Chart 1, IV-4:

After a normal pregnancy, this white male was delivered in 1953. He was first seen by a physician at the age of five months for an acute respiratory infection. At that time the patient was grossly retarded, small and delicate, having a large head. Polydactyly (six toes bilaterally), softening of the cranial bones, and "mild hypospadius" were also present. The patient had poor muscular tone and was lethargic; convulsive twitching were noted. An air ventriculogram demonstrated moderate ventricular enlargement with diffuse cortical atrophy, probably congenital. Apparently, blindness or deafness were not present. The patient developed poorly at home and died at the age of sixteen months with pneumonia. No autopsy was performed.

CASE THREE Chart 1, IV-3:

After an uncomplicated pregnancy and delivery, this white male was born November 11, 1949, weighing 6 lbs. 8½ oz. Congenital defects then noted included: (1) six toes on each foot and (2) an abnormal penis. A feeding problem the first two months, the patient later did well on Similac. He was subject to convulsions and was blind and deaf. At one year of age the patient was evaluated at Charity Hospital, New Orleans, Louisiana. He was retarded, had not sat up, crawled, or walked, and was usually apathetic and lethargic. Third degree hypospadius, enlarged head, six toes on each (containing phalanges), and softening of the skull bones were present. Complete blood counts, urine analysis, serological test for syphilis, alkaline phosphatase, non-protein nitrogen, and two cerebro spinal fluid examinations were considered normal. Electroencephalogram was interpreted as a normal waking test. Complete bone radiologic survey including bone age were not remarkable. Skull films demonstrated slight oxycephaly with increased intracranial markings. Films of the feet showed six toes bilaterally, each with phalanges. Chest roentgenograms revealed a boot shaped heart "suggestive of cardiac defect." During a pneumoencephalogram the patient ceased to breathe and expired. The diagnosis: LMBB syndrome. No autopsy was performed.

Examination of pedigree Chart 1 reveals that the proband, Figure 1, had three siblings, two males exhibiting features characterizing the LMBB syndrome and reported in this investigation as CASE TWO, IV-4, and CASE THREE, IV-3, respectively. The only normal and surviving sibling in this family is the eldest, a female, IV-2, born February 25, 1948.

The mother, III-3, age 30, has no history of radiation, lues, or rubella during pregnancy. There is no history of abortions. The father, III-6, age 35, had mumps when eight years old not complicated by orchitis. He also stated that he and his sister, III-7, had supernumerary toes which had been removed at the time of birth. The paternal grandfather, I-1, also had extra toes on each foot. There is no consanguinity in this pedigree. On the maternal side a

number of individuals have diabetes mellitus and have children with this condition. To keep the pedigree simple the children of these individuals were not shown. However, of the three women having diabetes mellitus, II-9 has one daughter and three sons: one son has diabetes. II-10 has two sons and three daughters: two of the daughters have diabetes. II-11 had no children and died from cancer of the colon.

Because of the mental deficiency encountered in the LMBB syndrome, one other bit of information might be pertinent. II-5 died in a mental institution. We were unable to ascertain the reason for her institutionalization. Of interest also are the abortions present in this kindred.

The major similarities and differences among the three brothers exhibiting LMBB syndrome in this pedigree are summarized in Table 1.

CASE FOUR (5699-I) Chart 2, II-9:

This white female, born June 10, 1918, was first seen at John Sealy Hospital in 1952. Her chief complaint was deafness in the right ear with recurrent bilateral otitis media for twenty years. All her life vision had been "hazy" and she was totally blind at night. Although she could see well enough to reach the 5th grade at school, at this time she could only distinguish light from darkness. Very large most of her life, she had been placed on a reducing diet and thyroid medication eight years before. At birth she had six fingers and six toes bilaterally; the extra digits were excised in infancy. Constipation had always been a problem. After menarche occurred at age 13, she had irregular menstrual periods associated with dysmenorrhea. However, eight years ago, because of a "positive blood test," forty-eight intra-muscular hip injections were administered on a weekly basis followed by cessation of dysmenorrhea and establishment of regular menses. Since age 29, occasional staggering episodes with a sensation of falling occurred.

On physical examination, temperature, pulse, and respirations were within normal limits. Blood pressure was 140/80–110. Height was 67 inches, and weight was 203 pounds. In appearance she was a large female, presenting a "buffalo obesity." Although generally mentally alert, she was somewhat slow in answering questions. Her skin was dry and roughened.

Pupillary reactions, extra ocular movements, and corneas were negative on eye examination. On fundoscopic visualization, the discs appeared pale and vessels were atrophied. There was considerable retinal pigmentation particularly on the medial side of the optic discs bilaterally. On visual testing, the patient could not perceive even large objects and was able only to detect variations in light intensity. The right tympanum was thickened, white, and contained three perforations through which serosanguineous fluid exuded. The left tympanum was thickened and dull but not perforated. There was a large pad of fat over the lower cervical and upper thoracic spine. The breasts were large, pendulous, and firm. The abdomen was rounded, large, with apron-type fat. Examination of the genitalia revealed a small clitoris and narrow vagina; the uterus and adnexae were not palpable. Small healed scars were evident on the ulnar sides of the fifth fingers and the lateral sides of the fifth toes bilaterally. The remainder of the physical examination, including neurological evaluation, was within normal limits.

Laboratory work included several complete blood counts which were unremarkable and a urine analysis showing a trace of albumin and 15–20 white blod cells/hpf. On serological test the Kolmer was positive 1:160 and the Kahn was doubtful. Radiological studies demonstrated bilateral mastoiditis, normal skull, and broad shortened 5th metatarsals in both feet.

A few months later the patient was hospitalized for mastoidectomy on the right. Audiogram revealed a marked right side hearing loss; a BMR was reported as -8; and serologically, the Kolmer was 1:320 and Kahn positive. The patient was placed on a reducing diet (weight then was 226 pounds) and on Penicillin—600,000 units daily for 12 days to eradicate any luetic activity. The mastoiditis improved markedly so that the patient was discharged without surgery.

In 1955, at the age of 37, the patient was again hospitalized for two weeks, having had recurrence of mastoiditis, not influenced by further Penicillin therapy. Weight was 216½ pounds and examination not remarkably different from previous ones except the skin now seemed smooth and moist. The VDRL was now negative. A right modified radical mastoidectomy was performed and after an uncomplicated convalescence, the patient was discharged. She was not seen again until this study was undertaken. At this time she was examined and personally interviewed by Dr. Kniker. Additional history revealed that there were three individuals with diabetes mellitus in this pedigree (Chart 2) II-3, a known diabetic since 1931, also had weak eyes originating from measles in childhood. II-7 died in 1930 at age 17. She was diagosed as having diabetes and Bright's disease of both kidneys in 1929. III-16, a 14-year-old female, was shown to have diabetes mellitus in 1953.

According to our informant, I-1 died at age 67 from pneumonia and I-2 died at age 72 from malignant melanoma. II-6 died at age 32 from sudden heart failure. She states that he had "the disease where the white blood cells eat the red blood cells" and this went away but came back again. A twin sister II-8, of the proband died of membranous croup when she was one month old. Another sister of the proband II-11 had polydactyly (six fingers on each hand), was sickly, and had a greatly enlarged liver. She lived only one month. II-13 has stomach ulcers and had a partial gastrectomy. His wife II-14, has heart trouble and is anemic. While attending school, in the third grade, it was discovered that III-3 was congenitally blind in one eye. III-12, now age 11, had convulsions and an enlarged heart at three weeks of age. III-17, as an infant, fainted readily and was anemic. She is now ten years old and apparently well. Of interest also in this pedigree is a miscarriage, II-12, and a stillbirth, III-1.

CASE FIVE (42854-M) Chart 3, III-1:

This negro female, born April 9, 1947, was first seen in July 1957, in the Out-Patient Clinic of the Children's Hospital with the complaint of "kidney trouble." For the past three years she had had intermittent urinary frequency, dysuria, and occasionally bloody urine. During the initial illness, which lasted a month, edema of the face and ankles occurred. In addition, the eyelids were always "droopy"; the head had to be held back for her to look forward. Although small objects could not be seen, she was able to read. Side-to-side

TABLE 1

Physical Findings in Three Brothers with Laurence-Moon-Bardet-Biedl Syndrome

Physical Findings	EXTENT OF INVOLVEMENT		
	Case 1 (IV-5)	Case 2 (IV-4)	Case 3 (IV-3)
Physical and Mental Retardation	+	+	+
Skull Findings:			
Sella Turcica (x-ray)	Normal	—	Normal
Cranial Vault and Brain	Enlarged skull, hydrocephalus, cortical thinning	Enlarged skull, hydrocephalus, cortical atrophy	Enlarged skull with oxycephaly and increased intracranial markings (x-ray), probable hydrocephalus
Pseudocraniotabes	+	+	+
Convulsions	+	+	+
Electroencephalogram	Impaired activity over left hemisphere	—	Normal
Eyes	Blind, bilateral nystagmus, external strabismus and colobomo of left eye	Normal	Blind
Hearing	Apparently normal	—	Deaf
Heart	Cardiomegaly, prominent systolic murmur and cyanosis	—	Boot-shaped cardiac x-ray shadow "suggestive of defect"
Genitourinary Abnormalities	Third degree hypospadius, bladder neck contracture	Mild hypospadius	Third degree hypospadius
Polydactyly	Toes and fingers bilaterally with syndactyly between toes	Bilaterally, involving toes	Bilaterally, involving toes
Age and Cause of Death	6 months—volvulus of intestine due to malrotation	16 months—pneumonia	12 months—during pneumoencephalogram

oscillations of both eyes had been noted. Dyspnea occurred on exertion, but there was no cyanosis. Nocturia occurred several times nightly. The parents and five younger siblings were said to be in good health. The maternal grandmother has "heart trouble" of recent origin; no other significant illnesses were reported in relatives. Prenatal period was uncomplicated. Delivered by a mid-wife, the patient was "blue" for the first ten minutes of life. A sixth toe on the left foot was discovered then. The child does not "get along" with other children well, and in three years of schooling has made consistently unsatisfactory grades.

Physical examination at the first visit revealed a 10-year-old well-developed and slightly obese female, in no distress. Temperature, pulse, and respirations were within normal limits. Height was 144.8 cm. (75–90 percentile for age ten); weight 96.5 pounds (90–97 percentile for age ten). The facies appeared dull. The skin was roughened, dry, and in areas, scaly. There was bilateral ptosis of the upper eyelids and marked lateral nystagmus of the left eye. The breasts were beginning to develop. There was no cardiomegaly clinically. A harsh grade thre systolic murmur was heard best in the third and fourth interspace left of the sternum; a diastolic component was questionably present. The external genitalia were somewhat small and prepubertal. A sixth toe, containing bone, was present laterally on the left foot. Neurological examination was, excluding nystagmus, entirely negative. She was cooperative, but able to say only "Yas suh" or "No suh." The Ophthalmology Department found vision in the right eye to be 20/30; in the left eye, 20/40. On horizontal gaze, nystagmus was marked in the left eye, with the fast component in a lateral direction. Otherwise, extra-ocular movements were intact. On confrontation there was "narrowing" of the lower portion of the fields (ptosis?). Intraocular tension was normal. Following refraction, visual acuity was unimproved. Bilaterally, there were diffuse gray heavily pigmented areas over the retinal surfaces. The optic nerve on the left seemed pale and atrophic. There was generalized narrowing of retinal vessels.

During the ensuing months, many laboratory studies were performed. Complete blood counts were always within normal limits and serological test for syphilis was negative. The initial urine specimen contained large numbers of white blood cells; after gantrisin therapy for several weeks, the urine analysis was normal. The urinary ferric chloride test was negative. Chest films demonstrated an enlarged, globular heart. An enlarged pulmonary trunk with increased pulsations; a slightly enlarged right ventricle and atrium were discovered on cardiac fluoroscopic examination. This was interpreted as being consistent with a left to right shunt, probably interatrial septal defect. An electrocardiogram substantiated this with indications of marked right atrial enlargement and a grade one A-V block.

In October the child received a complete psychometric evaluation in our Child Guidance Clinic. She was consistently low in all areas, attaining a full scale I. Q. of 54 on the Wechsler Intelligence Scale for Children. Her emotional maturity was on an equally low level, and it was the examiner's opinion that the marked mental deficiency stemmed from organic impairment. She was seen at the end of October because of a chalazion of the right eye which rapidly healed under conservative management.

The patient was hospitalized five days in December 1957, on the Orthopedic Service. The supernumerary digit containing two phalanges and metatarsal segment (the fifth metatarsal was bifid) was excised. At this time breasts were moderately developed and pubic and axillary hair were present. Menarche had not occurred. A clitoris could not be distinguished, nor could labia minora. The hymen was quite small and thick with a few tiny perforations. Serum electrolytes and calcium; blood fasting sugar and NPN; urine analysis; ferric chloride urinary test, were negative. Excretory urograms suggested right pyelonephritis; there was some enlargement of the right renal pelvis and bilateral blunting of calyces. Residual urine collection was negligible. Addis and Esback 12-hour urine tests were negative. At cystoscopic examination on December 19, 1957, both uretral orifices were abnormally close to the midline situated on hillocks, preventing passage of catheters for retrograde studies. The impression was bilateral ureteral stenosis—ureteral urine was negative for acid fast organisms by culture.

Gynecological consultation was obtained on December 20th—no evidence of a vaginal opening was located; on rectal examination an infantile uterus was questionably palpated.

The patient was due to have Orthopedic, Urologic and Gynecologic follow-up examinations in January, but did not return.

An investigation into the genetic history of the family, Chart 3, revealed that I-1 has diabetes mellitus. III-2, the sister of the proband, was found to locomote by "scooting" around on the floor. Further information obtained revealed that this negro female was delivered on October 9, 1950, after a full-term uncomplicated pregnancy. Marked cyanosis persisted a few hours, and no crying occurred until the second day.

A physical examination, in April 1958, revealed a small retarded microcephalic female who spoke random meaningless words and was not hyperactive. Weight—33½ pounds (50 percentile for 4 years); length—42 inches (50 percentile for 4 years); and head circumference—47.5 cm. (3 percentile for 3 years). There was bilateral internal strabismus, with no evidence of retinitis pigmentosa. High arched palate. She could not bear weight or maintain balance on legs adequately. All muscle stretch reflexes were hyperactive, more so on left lower extremity where a positive Babinski was present. There was a spastic gait, when supported, and muscles of lower extremities were atrophied.

CBC, urinalysis, STS, were negative.

Psychological testing was unsatisfactory due to inability of patient to cooperate; she demonstrated echolalia, perseveration, no conceptual thinking. The impression was severe mental retardation.

She is now awaiting placement in the Austin State School.

Discussion

The present report deals with the available cases of LMBB syndrome reported since Burn (9) reviewed the literature in 1949. These constitute approximately one-half of the reported

cases; the rest, mainly reported in the foreign literature, were unavailable for analysis.

The incidence of the individual components of the LMBB syndrome reported in 34 publications and those reported by us are summarized in Table II. A total of 65 affected individuals are reported in 47 families. This number constitutes 39 males (60%) and 26 females (40%). Obesity was reported in 54 patients, 31 males and 23 females. Mental deficiency occurred in 52 patients, 30 males and 22 females. Polydactyly and/or syndactyly were present in 49 patients and were distributed as follows:

Males	*Females*	*Polydactyly*	*Syndactyly*
15	4	Hands and Feet	One male, both feet
6	1	Hands only	Three males, both feet
6	6	Feet only	*
1	1	Left hand	One male, both feet
–	3	Left foot	
1	–	Right foot	
1	–	Left hand and left foot	
–	1	Right hand and right foot	
1	–	Left hand and both feet	
1	–	Right hand and both feet	
–	1	Both hands and right foot	
—	—		
32	17		

* Three females; one, hands and feet; one, both feet; one, left foot.

In addition, syndactyly alone was present in two females, feet only. Forty-four patients, 25 males and 19 females, had retinitis pigmentosa. Genital dystrophy was reported in 39 patients, 28 males and 11 females. It was familial in 31 individuals, 20 males and 11 females. Seventeen of the affected patients, 7 males and 10 females, from 13 families, came from consanguineous marriages. The percentage incidence of the individual components are shown in Graph 1.

Graph 2 shows the distribution of 65 cases of LMBB syndrome according to number of cardinal features present. In 49 cases (75%), the basis for diagnosis was the presence of at least four of

TABLE 2

Incidence of Individual Components of Laurence-Moon-Bardet-Biedl Syndrome in Sixty-Five Cases Reported From 1949 to 1956 Including the Present Study

Date of Publication	Author	Sex of the Affected	Consanguinity	Number of Families Reported	INDIVIDUAL COMPONENTS: Obesity	Genital Dystrophy	Retinitis Pigmentosa	Mental Deficiency	Polydactyly	Familial	Additional Family History
1949	Montanare and Garrote (29)	M	....	I	+	+	+	....	....	....	
		M	....	II	+	+	....	....	....	....	1 older male sibling has a similar condition
		M	....	III	....	....	+	+	+	....	
1950	Csorba (12)	F	....	....	+	+	+	+	+	+	
		M	....	....	+	+	....	+	+	+	1 normal male sibling; mother had 2 molar pregnancies
		M	....	....	+	+	+	+	+	+	
1950	Cunningham and Raymond (13)	M	....	....	+	....	+	+	+	....	4 normal siblings (sex not stated)
1950	Francke (15)	F	+	I	+	....	+	+	+	+	Not reported
		F	....	....	+	....	+	+	+	+	
		M	....	II	+	+	+	+	....	....	An older male and an older female sibling are deceased and both showed similar conditions
1950	Keifer (23)	F	....	I	+	....	+	+	+	....	Not reported
		F	....	II	+	....	+	+	+	....	1 male sibling had impaired vision and polydactyly
		F	....	III	+	....	+	+	+	....	1 male sibling has polydactyly and is blind and obese; 1 male sibling has polydactyly and is mentally deficient, blind and obese
1950	Rao (36)	M	....	....	+	+	+	+	+	+	2 male siblings (1 died at a year of age with smallpox); 5 female siblings (1 had polydactyly and died at 2 years of age)
		F	....	....	....	....	....	....	+	+	
1951	Bisland (6)	M	....	....	+	+	+	+	+	....	1 male sibling; 2 female siblings
1951	Hertzberg (21)	F	+	....	+	....	+	+	+	....	1 normal male sibling
1951	Millichap (28)	M	+	....	+	+	+	+	+	....	1 normal male sibling; 1 female sibling (stillbirth—had polydactyly)
1951	Naylor (31)	M	....	....	+	+	+	+	+	....	3 normal male siblings; 1 normal female sibling
1951	Olaya (32)	M	....	....	+	+	+	....	+	....	Not reported
1952	Bornstein (7)	M	+	....	....	....	+	....	+	....	4 normal male siblings; 2 normal female siblings
1952	Gantayat (19)	M	+	....	+	+	+	+	+	....	1 normal male sibling; 1 normal female sibling
1952	Kessel (24)	F	....	....	+	....	....	+	+	....	1 normal male sibling
1953	Agarwal (1)	F	....	....	+	+	+	+	+	....	10 siblings—all deceased (sexes not stated)
1953	Grebe (20)	F	+	I	+	+	+	+	+	....	5 normal male siblings; 1 normal female sibling; 1 affected male sibling (deceased) and 1 affected female sibling (deceased)
		F	....	II	+	+	....	+	....	....	7 normal female siblings; 2 normal male siblings
1954	Bergman (3)	M	....	....	+	+	+	+	+	....	Not reported
1954	Francois (16)	F	+	....	+	+	....	+	....	....	1 male sibling with an eye condition
1954	Galmozzi (18)	M	....	....	+	+	....	....	+	....	1 normal female sibling

Incidence of Individual Components of Laurence-Moon-Bardet-Biedl Syndrome in Sixty-Five Cases Reported From 1949 to 1956 Including the Present Study

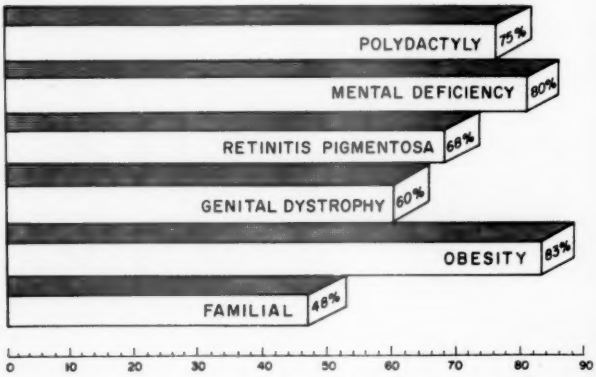
INDIVIDUAL COMPONENTS

1954	Francois (16)	F	+	....	+	+	....	+	....	....	1 male sibling with an eye condition
1954	Galmozzi (18)	M	....	....	+	+	....	+	+	....	1 normal female sibling

Incidence of Individual Components of Laurence-Moon-Bardet-Biedl Syndrome in Sixty-Five Cases Reported From 1949 to 1956 Including the Present Study

Date of Publication	Author	Sex of the Affected	Consanguinity	Number of Families Reported	INDIVIDUAL COMPONENTS: Obesity	Genital Dystrophy	Retinitis Pigmentosa	Mental Deficiency	Polydactyly	Familial	Additional Family History
1954	O'Mahony (33)	F	....	....	+	+	+	+	....	+	1 normal male sibling; 1 normal female sibling
		M			+		+	+		+	
1954	Rosemblit (40)	F			+	+	+		+		8 normal male siblings: 5 are deceased
1954	Venezia, Romeo and Gallen (46)	M	+	....	+	+	....	+	+	....	1 normal male sibling; 2 males (twins) were stillborn
1955	Fischer (14)	M	....	....	+	....	+	+	+		Not reported
1955	Frezzotti and Biagiani (17)	M	....	I	+	+	+	+	+	+	Not reported
		M	....		+	+	+	+	+	+	Not reported
		F		II	+		+	+	+		
1955	Mosberg (30)	M	....	....	....	....	....	....	+	+	
		M	....	....	....	....	....	....	+	+	
		M	....	....	....	....	....	....	+	+	Not reported
		F	....	....	....	....	....	....	....	+	
1955	Todd (45)	M			+	+	+	+	+		4 normal male siblings; 5 normal female siblings
1955	Wolff and Etzine (48)	M	....	I	+	....	+	+	+	+	Large family—number of siblings not reported
		M	....		+	....	+	+	+	+	
		F	....	II	+	....	....	+	....	+	5 normal siblings (sexes not stated)
		F			+			+		+	
1955	Wolski (49)	M	....	....	+	+	+	....	+	....	Not reported
1956	Bimbi and Kovacs (5)	M	....	....	+	+	....	+	+	+	2 female siblings (1 has polydactyly); 1 male sibling with polydactyly died at 14 days of age; 1 sibling (sex unknown) with polydactyly died at birth
		M	....	....	+	+	....	+	+	+	
1956	Kalbian (22)	M	+	..	+	+	+	+	+	....	Not reported
1956	Kuromaru (25)	F	+	I	+	+	+	+	+	..	1 normal male sibling
		F	+	II	+	+	+	+	+	+	
		M	..		+	+	+	+	....	+	Not reported
		F	+	III	+	....	+	+	....	....	
1956	Ross (41)	M	....	....	+	+	+	+	+	....	1 normal female sibling; 1 sibling (sex unknown) died a half hour after birth
1956	Siegler (42)	F	....	....	+	....	+	+	....	+	2 normal female siblings
		F	....	....	+	....	+	+	....	+	
		M			+				+	+	
1956	Wu (50)	M	....	....	+	+	....	+	....	+	1 normal male sibling; 1 normal femlae sibling
		M	....	....	+	+	....	+	....	+	
			Present Study								
		M	....	..	....	+	....	+	+	+	1 normal female sibling; father and great grandfather had polydactyly
		M	....	I	....	+	....	+	+	+	
		M	....	..	+	+	..	+	+	+	
		F	....	II	+	+	+	....	+	+	2 normal male siblings; 1 abortion; 5 female siblings (2 with diabetes and 1 with oplydactyly)
		F	....	III	....	+	+	+	+	+	

PERCENTAGE INCIDENCE OF INDIVIDUAL COMPONENTS OF LMBB SYNDROME IN 65 CASES

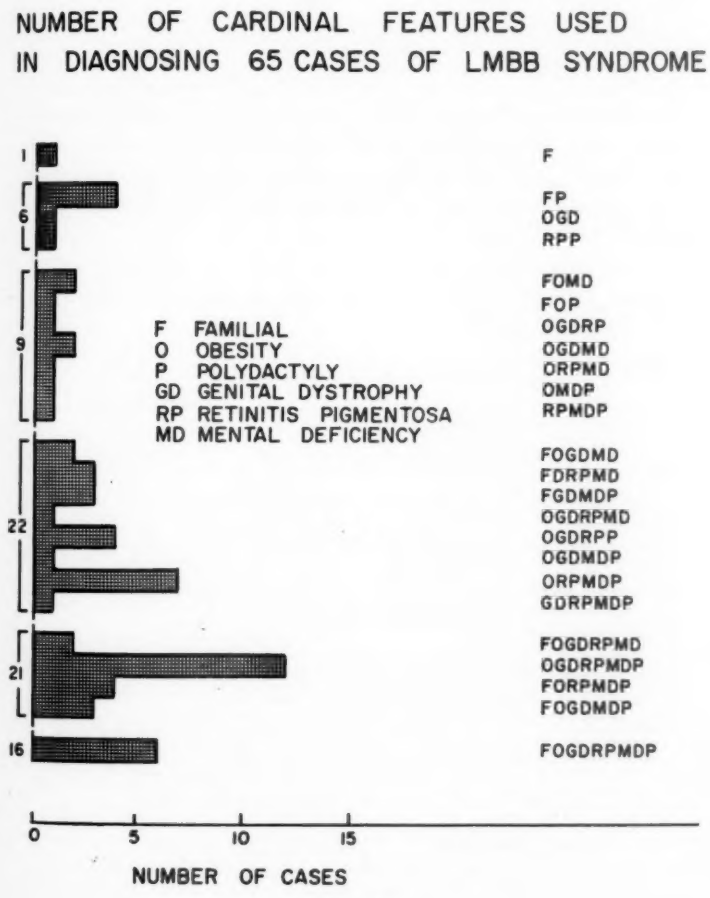


GRAPH 1.

the cardinal features, if "familial" is included as one of the features characterizing this syndrome. This leaves 16 cases (25%), which were diagnosed on the presence of one to three signs. According to Reilly and Lisser (37), "familial" is a *sine qua non* if all of the classical syndrome is not present. However, to demand the presence of any particular feature in this syndrome is hard to justify. In any gene determined syndrome there is considerable variation in expressivity between affected individuals and between certain families. Table I demonstrates this variation of signs in a family with three affected males. The numerous associated anomalies, such as ocular, skeletal, central nervous system and metabolic disturbances reported in cases of LMBB syndrome attest to the considerable variability one would expect. In fact, it would be rare from a genetic point of view for the physician to find all of the cardinal features present in all affected individuals. The gene or genes responsible for any syndrome exert their action in a different gene environment in each affected person. The action of modifying genes may also give rise to other variations of the syndrome. Burn (9) believes that deaf mutism is not merely a chance association, but is related to the syndrome in

some manner. It may very well represent another of the pleiotropic actions of the genome.

The unexplained excess of males and the high incidence of consanguinity encountered in cases of LMBB syndrome in previous reviews of the literature (9, 10, and 43) and adequately discussed by the authors, closely parallel those of the present review. Thus the elucidation of the actual mechanism responsible



GRAPH 2.

for the LMBB syndrome remains enigmatic and must be found by determining the role of the gene or genes in the metabolic processes in these patients and their families. This, of necessity and from a purely technical aspect, will be tedious and time consuming. In the meantime, to our knowledge, Kalbian's (22) pedigree of a case of LMBB syndrome in an Arab boy is the best on record to lend support to the now generally accepted theory of a recessive type of inheritance in this syndrome.

Summary

1. The literature on the LMBB syndrome has been searched and 34 publications have been reviewed. The findings closely parallel those of previously published reviews.

2. Five cases of this syndrome, with necropsy findings on one of these, have been reported. This included a family group with three affected males.

3. Possible reasons for the great variation in the so-called classical picture of the LMBB syndrome have been discussed from a genetic point of view.

REFERENCES AND NOTES

1. Agarwal, L. P.: Laurence-Moon-Biedl Syndrome. *J. of The Indian Medical Association*, 22: 467–468, 1953.
2. Bardet, G.: Sur un syndrome d'obesite infantile avec polydactylie et retinite pigmentaire. *Univ. Paris Thesis*, 470: 107, 1920.
3. Bergman, B., and A. N. Eden: Laurence-Moon-Biedl Syndrome. *J. of Pediatrics*, 45: 603–605, 1954.
4. Biedl, A.: Geschwisterpaar mit adiposa-genitaler dystrophie. *Deut. Med. Wochens.*, 48: 1630, 1922.
5. Bimbi-Kovaes, A. E., and A. F. Hardyment: Potter's facies associated with polycystic kidney, with Laurence-Biedl-Moon Syndrome in siblings. *Canad. M. Ass. J.*, 74: 549–551, 1956.
6. Bisland, T.: The Laurence-Moon-Biedl Syndrome. Report of a typical case with complete necropsy. *Am. J. Ophth.*, 34: 874–884, 1951.
7. Bornstein, M. B.: Aniridie Bilaterale avec polydactylie. Relations des anomalies Oculaires du type colobomateaux associees a des malformations squelettiques avec les formes atypiques du syndrome de Bardet-Biedl. *Journal de Genetique Humaine*, 1: 211–226, 1952.
8. Brattgard, S. O.: The Pathology of Laurence-Moon-Biedl Syndrome. *Acta path. et microbiol. Scandinav.*, XXVi: 525–537, 1949.
9. Burn, R. A.: Deafness and the Laurence-Moon-Biedl Syndrome. *Brit. J. Ophth.*, 34: 65–88, 1950.
10. Cockayne, E. A., D. Krestin, and A. Sorsby: Obesity, hypogenitalism, mental retardation, polydactyly and retinal pigmentation. The Laurence-Moon-Biedel Syndrome. *Quart. J. Med., N. S.*, 4: 93–120, 1935.

11. Csik, L., and K. Mather: Sex incidence of certain hereditary traits in man. *Ann. Eugenics*, 8: 126–145, 1938.
12. Csorba, A: Laurence-Moon-Biedl Syndrome. Three Cases in one Family. *Confinia neurol.*, 10: 378–386, 1950.
13. Cunningham, J. A. K., and J. A. Raymond: Laurence-Moon-Biedl Syndrome. Report of a Case. *New Zealand Medical Journal*, 49: 417–419, 1950.
14. Fischer, E.: The Laurence-Moon-Biedl Syndrome. *J. of Irish Medical Association*, 37: 343–344, 1955.
15. Francke, C.: The gonads in the Laurence-Moon-Biedl Syndrome. *J. Clin. Endocrinol.*, 10: 108–112, 1950.
16. Francois, J.: A propos d'une famille presentant des anomalies oculaires du type colobomateux de puis la colobome unilateral de l'iris jusqu'a l' anophtalmie bilaterle associee au syndrome de Bardet-Biedl. *Journal de Genetique Humaine*, 2: 3–4, 1953.
17. Frezzotti, R., and R. Biagiani: Clinical and etiopathogenetic observation on the Laurence-Moon-Bardet-Biedl Syndrome. *Riv. Clin. pediat.*, 55: Sup. 1, 163–186, 1955.
18. Galmozzi, A.: Sindrome di Laurence-Moon-Bardet-Biedl. *IL Lattante*, 25: 669, 1954.
19. Gantayat, S. N., and R. Suryaprasadarao: Laurence-Moon-Biedl Syndrome. *J. Indian M. A.*, 21: 367–369, 1952.
20. Grebe, Hans: Contribution au diagnostic differential du syndrome de Bardet-Biedl. *J. De Genetique Humaine*, 2: 127–144, 1953.
21. Hertzberg, R.: The Laurence-Moon-Biedl Syndrome. *Med. J. Australia*, 1: 90, 1951.
22. Kalbian, V. V.: Laurence-Moon-Biedl Syndrome in an Arab boy: Familial Incidence. *J. of Clinical Endocrinology and Metabolism*, 16: 1622–1625, 1956.
23. Keifer, W. S., *et al.*: Laurence-Moon-Biedl Syndrome, A confused symptom complex. *Am. J. Obst. & Gynec.*, 60: 721–730, 1950.
24. Kessel, I.: The Laurence-Moon-Biedl Syndrome. *J. Pediat.*, 40: 96–100, 1952.
25. Kuromaru, S., and S. Usa: Three cases of Laurence-Moon-Biedl Syndrome. *Pol. psychiat. neur. jap.*, 10: 1, 1956.
26. Laurence, J. C., and R. C. Moon: Four cases of "retinits pigmentosa" occurring in the same family and accompanied by general imperfections of development. *Ophth. Rev., London*, 2: 32–41, 1866.
27. Macklin, M.: The Laurence-Moon-Biedl Syndrome, A genetic study. *J. Hered.*, 27: 97–104, 1936.
28. Millichap, J. G.: Laurence-Moon-Biedl Syndrome. *Proceedings of The Royal Society of Medicine*, 44: 1063–1064, 1951.
29. Montanave, J. C., and R. A. Garrote: Tres Nuevos Casos de Sindrome de Laurence-Biedl. *Semana med.*, 56: 671, 1949.
30. Mosberg, W. H. J.: The Laurence-Moon-Biedl Syndrome. *Bull. School Med. Univ. Maryland*, 40: 78–85, 1955.
31. Naylor, E. G.: Laurence-Moon-Biedl Syndrome in a Central African Native. *Brit. M. J.*, Vol. 1, 624, 1951.
32. Olaya, Restrepo M.: Historia de un caso de endocrinologia. *Rev. Fac. de med., Bogota*, 20: 89–99, 1951.
33. O'Mahony, P. F.: Laurence-Moon-Biedl Syndrome. *New England J. of Med.*, 251: 439–440, 1954.

34. Ornsteen, A. M.: A contribution to the pathogenesis and heredity of the Laurence-Moon-Biedl Syndrome (dystrophia adiposogenitalis, retinitis pigmentosa, mental deficiency and polydactylism); report of three cases in one family. *Am. J. Med. Sc.*, 183: 256–268, 1932.

35. Radner, S.: On Laurence-Moon-Biedl Syndrome. *Acta Medica Scandinavica*, 105: 141–152, 1940.

36. Rao, K. S.: The Laurence-Moon-Biedl Syndrome, Report of Two Cases. *Indian M. Gaz.*, 85: 542–544, 1950.

37. Reilly, W. A., and H. Lisser: Laurence-Moon-Biedl Syndrome. *Endocrinology*, 16: 337–357, 1932.

38. Rieger, H.: Ueber die erblichkeits—verhältnisse des Biedl-Bardetschen Syndroms. *Arch. F. Riss. Biol.*, 23: 298–300, 1931.

39. Rolleston, Sir Humphrey D.: The Endocrine Organs in Health and Disease. Henry Milford, London, 1936.

40. Rosemblit, E., and J. E. Segal: Sindrome de Laurence-Moon-Biedl con hipercorticoidismo. *Semana Med.*, 105: 840–847, 1954.

41. Ross, C. F., L. Crome, and D. Y. Mackenzie: The Laurence-Moon-Biedl Syndrome. *J. Path. & Bact.*, 72: 161–172, 1956.

42. Siegler, A. M., and E. Weisfogel: Laurence-Moon-Bardet-Biedl Syndrome, A Family Group with three Affected Siblings. *Obst. Gyn.*, 8: 332–335, 1956.

43. Sorsby, A., H. Avery, and E. A. Cockayne: Obesity, Hypogenitalism, Mental Retardation, Polydactyly, and Retinal Pigmentation. *Quart. J. Med. N. S.*, 8: 51–68, 1939.

44. Streiff, E. B. et C. Zeltner: Le Syndrome de Laurence-Moon-Bardet-Beidl, Etude Clinique et Genetique. *Arch. D'Ophtalm.*, 2: 289–340, 1938.

45. Todd, J.: A Case of the Laurence-Moon-Biedl Syndrome with Paranoid Psychosis. *Amer. J. Ment. Deficiency*, 60: 331–334, 1955.

46. Venezia, R., R. Romeo, and J. J. Galleg: Etude d' un cas de syndrome de Laurence-Moon-Bardet-Biedl. *Algerie Medicale*, 58: 491–495, 1954.

47. Warkany, J., G. S. Frauenberger, and A. C. Mitchell: Heredofamilial deviations. The Laurence-Moon-Biedl Syndrome. *Am. J. Dis. Child.*, 53: 455–469, 1937.

48. Wolff, J. E., and S. Etzine: A Report on Four Cases of Laurence-Moon-Biedl-Bardet Syndrome. *South African M. J.*, 29: 280–281, 1955.

49. Wolski, J.: Przypadek zesponu Laurence-Moon-Biedla ze zgrubieniem Kosci czolowej. *Polski. tygodnic lek.*, 10: 1470–1472, 1955.

50. Wu Hou-Chang: Laurence-Moon-Biedl Syndrome, Report of 2 Cases. *Chinese M. J.*, 74: 75–80, 1956.

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ORAL TREATMENT OF SUPERFICIAL MYCOSES WITH GRISEOFULVIN

J. Fred Mullins[a], Noel A. Jampolsky and Mary E. Pinkerton

Griseofulvin was first isolated as a fermentation product of *Penicillium griseofulvum* in 1939 by Oxford, Raistrich and Simonart (6); and later from other species of *Penicillium* (2) Investigations by Brian *et al* (3) have shown griseofulvin to be of value as a fungistatic agent in plant infections. In 1958, Gentles reported the successful use of oral griseofulvin for experimental *Trichophyton* and *Microsporum* infections of guinea pigs (4). Later, Lauder and O'Sullivan (5) cleared *Trichophyton verrucosum* infections in calves with this agent. Recently Williams *et al* (8) in England and Blank and Roth (1) in the United States published the first reports on the efficacy of oral griseofulvin on dermatomycoses in man.

Since many patients fail to respond to present topical fungicides, it has long been felt that a systemic medication would be exceedingly valuable for the management of superficial mycoses and perhaps the therapy of choice. The purpose of this study was to determine the effect and toxicity of orally administered griseofulvin in cases of resistant superficial mycoses.

Methods and Materials

There was a total of 24 cases, varying in ages from 2½ to 70. Eleven were males and 13 were females. There were 17 patients with *Trichophyton rubrum* infections. Of these, 9 were generalized, 7 were limited to groin and buttocks, 3 patients had nail involvement and one had generalized and deep pustular lesions. Of the 7 children with tinea capitis 2 had *Trichophyton tonsurans* infections, 4 *Microsporum audouini* and 1 *Microsporum canis*. Most patients in this series had been previously treated unsuccessfully with topical therapeutic agents.

Prior to therapy, cultures were made from the skin of all patients and preparations cleared in KOH for microscopic examination. Also, complete blood counts, liver function tests, urinalyses and photographs were taken. Whenever possible the patients were seen at weekly intervals and repeat KOH's, cultures and complete blood counts were done. Liver function tests, urinalyses and photographs were repeated at the completion of therapy. Three patients were hospitalized during the course of treatment for closer observation.

Griseofulvin was administered orally in daily dosages of 1 gm. to adults

TABLE 1

Summary of Cases

Case	Age	Sex	Race	Diagnosis by Culture	Yrs. of Inf.	Dosage and Frequency	Cult. Neg. Wks.	KOH Neg. Wks.	Therapy Wks.	Clinical Results	Untoward Reactions	Follow-up Wks.	Comments
1	70	F	W	Gen. pustular T. rubrum and onychomycosis	5	lgm/d	2	3	5½	Clear	Acute hypertension 38th d. Stopped drug	6	Pruritus ceased 3 d. Nails continued normal regrowth.
2	50	F	N	Gen. T. rubrum	4	lgm/d	1	1	4½	Clear	None	6	Pruritus ceased 4 d.
3	58	F	N	Gen. T. rubrum	14	lgm/d	2	3	7	Clear	Transient headache during 2nd wk.	4	Continues clear
4	37	F	N	Gen. T. rubrum	4	lgm/d for 7 d. Stopped 4 d. and resumed	3	4	5	Clear	None	b	Allergic to penicillin. Pruritus ceased 3 d. Clear
5	42	F	N	Gen. T. rubrum	5	lgm/d	4	3	5	Clear	WBC 4700→5900 PMN 79%→53% Lymph 20%→45%	6	WBC & Diff. returned to normal. On 4th followup wk. a positive culture between toes. Buttocks positive on 6th wk. Clinically clear
6	67	M	LA	T. rubrum of groin	6	lgm/d	1	2	3	Clear	None	7	Pruritus ceased 3 d.
7	51	M	LA	T. rubrum, groin and buttocks	6	½gm/d for 2½ d. Stopped 30 d., then lgm/d.	+6	+6	6	Cleared Recurred	None	c	Failure to date
8	9	M	W	t. capitis M. audouini	1.5 mo.	750mgm/d	4	+8	8	Complete regrowth	None	1	Some hairs show + Wood's light with mycelial elements at tips only. Neg. culture
9	6	F	W	t. capitis t. tonsurans	1 yr.	250mgm/d	4	4	7	Hair regrowing	None	1	Hair regrowing with + Wood's light. KOH and culture neg.
10	9	F	LA	t. capitis t. tonsurans	1	500mgm/d	2	2	5	Normal	None	4	Continues clear
11	10	F	W	t. capitis M. audouini	6–8 mos.	375mgm/d (750mgm/d during 3rd wk.)	4	4	5	Normal growth	None	..	On 5th wk., fluorescence doubtful. KOH neg. Dosage accidentally doubled in 3rd wk.
12	6	M	W	t. capitis M. audouini	6–8 mos.	250mgm/d (500mgm/d during 3rd wk.)	5	5	5	Scalp clear	None	..	Dosage accidentally doubled during 3rd wk.
13	22	M	W	T. rubrum of groin	1	lgm/d	a	+1	1	Slight improvement	None	c	Mild pruritus at 7 d.

TABLE 1—Continued

TABLE 1—Continued

Summary of Cases

Case	Age	Sex	Race	Diagnosis by Culture	Yrs. of Inf.	Dosage and Frequency	Cult. Neg. Wks.	KOH Neg. Wks.	Therapy Wks.	Clinical Results	Untoward Reactions	Follow-up Wks.	Comments
14	64	F	N	Gen. T. rubrum	6	lgm/d q. 3 d.	2	2	2	Clear	WBC 7200→6050 PMN 77%→53% Lymph 16%→41% (In 2nd wk.)	5	Pruritis ceased 5 d. Continues clear. WBC count and differential returned to normal 2nd post-treatment wk.
15	51	F	N	T. rubrum of buttocks	7	lgm/d q. 3 d.	3	3	3	Clear	None	2	Never pruritus. Penicillin allergy Continues clear.
16	39	F	N	T. rubrum of buttocks	Unknown	lgm/d for 7 d. Skipped 2 wks. then lgm/d q. 3 d.	3	+4	4	Scaling persists at border	None	b	Treated for 1 wk. Failed to return for 3 wk. at which time culture was neg. but KOH pos.
17	33	M	LA	T. rubrum, groin and buttocks	2	lgm/d q. 3 d.	+2	2	2	Slight improvement	None	c	Pruritus decreased but still present.
18	62	M	W	T. rubrum, onychomycosis	Unknown (yrs.)	lgm/d q. 3 d.	5	7	7	Clear	None	c	t. manuum clear at 7 wks. Nails show early normal growth.
19	24	M	W	T. rubrum, groin	4	lgm/d q. 3 d.	1½	1½	1½	Clear	None	1	Pruritus ceased in 3 d. Continues clear. Lesion was a solitary area.
20	53	F	W	Gen. T. rubrum	8	lgm/d for 3 d. then lgm. q. 3 d.	a	+2	2	Decrease scaling and erythema	None	c	Pruritus ceased in 3 d.
21	55	M	W	Gen. T. rubrum with onychomycosis	36	lgm/d for 3 d. then lgm. q. 3 d.	+1	+1	2	No change	None	c	Cessation pruritus 4 d.
22	7	M	LA	t. capitis M. canis	1 mo.	250mgm. q. 3 d.	+2	+2	2½	No change	None	c	Malignant reticuloendothelioses. No change of WBC after 18 d.
23	40	F	LA	Gen. T. rubrum	10	lgm/d for 3 d. then lgm. q. 3 d.	+1	2	2	Decrease scaling and erythema	None	c	Cessation of pruritus in 3 d.
24	2½	M	N	t. capitis M. audouini	4	250mgm. q. 3 d.	4	6	6	Hair regrowth	WBC 11,350→10,700 PMN 50%→20% Lymphs 42%→59% Eos 7%→19%	10 days	Over next wk. total WBC remained same but still has 20% PMN's, 67% lymphs and 5% Eos. Continuing to normal level in next 3 d.

a Too soon for growth.
b Failed to return.
c Still on therapy.

and 14 mgms./kg. to children. This dosage schedule was later reduced in an attempt to determine minimal effective therapeutic range.

Discussion of Results

The results of our observations of 24 patients are summarized in Table 1. In our cases of *T. rubrum* infections, the cultures became negative in from 1 to 4 weeks with an average of 2 weeks, although the KOH often remained positive for an additional week. However, because of a persistent mild to moderate scaling in most patients even after the KOH became negative, treatment was continued for an additional period of several weeks.

Case number 1 was unusually interesting in that she had a generalized deep pustular infection as well as onychomycosis. After 5½ weeks of therapy on 1 gm./day she was clinically and culturally free of infection with the exception of her nails. Because of an acute hypertensive episode, the griseofulvin was discontinued, although it was known that such episodes had antedated our therapy. Her blood pressure soon returned to normal but therapy was not reinstituted and during the following 6 weeks she remained clear and her nails continued to grow out normally.

It was noted that in practically every patient pruritus ceased in 3 to 5 days. From our earlier observations, it seemed likely that we were giving more griseofulvin than was required to clear the infection and so variations in our dosage schedule were instituted. On 1 gr. every third day, complete clearing was noted in cases 14, 15 and 19 within 2 to 3 weeks and yet case 17 showed continued pruritus at 2 weeks and case 18 required 7 weeks to clear his tinea manuum.

In case 7, after a total of 2.5 gms. administered over 5 days, there was complete clearing in 3 weeks, but during the fourth week infection recurred in two small areas. Despite 1 gr./day for the next 7 days the KOH and culture remained positive. This was the only definite recurrence in our series, but in case 5, a positive culture of *T. rubrum* was grown from between the toes on the 4th post-treatment week, and from the buttocks on the 6th week although these areas were clinically clear. Further follow-up is required on this patient to determine if clinical lesions will recur.

From these results we are as yet unable to arrive at any definite conclusions about the minimal effective dosage.

In our cases of tinea capitis, the results have so far been identical for both *Trichophyton* and *Microsporum* infections. Usually, no changes were noted until the third week, at which time, the Wood's light revealed a normal nonfluorescent zone at the proximal portion of the hair. From Figure I it can be seen that a relatively sharp line of demarcation exists between normal and infected portion of the hair. With continued therapy the infection is mechanically displaced by healthy new growth. Figures III through VI show typical clinical improvement in 2 cases.

In case 8 the culture reverted to negativity by the fourth week, but during four weeks of continued therapy and several weeks

FIG. I. Hair with line of demarcation between involved and uninvolved portion five weeks after treatment. Mag. × 23.

FIG. 2. Tip of hair of Figure I showing endospores of *T. tonsurans*. Mag. × 150.

post-treatment, he has continued to exhibit a positive Wood's light examination in the small hairs previously infected with *M. audouini*. The KOH examination reveals that these hairs have many mycelial elements within the shafts down to a sharp point of demarcation with the normally growing hair. No spores are seen and repeated attempts to culture the fungus from these hairs have proven them sterile.

A fluorescent zone was noted in several other patients at the base of the newly formed hairs, but in these patients repeated KOH examinations failed to reveal any fungus element and it

was felt that the false positive fluorescence was provided by a keratin ring at the junction of the hair with the scalp (*7*).

From these observations we feel that neither the Wood's light nor the KOH should be used as the only criterion of clearing, and that repeated cultures are necessary.

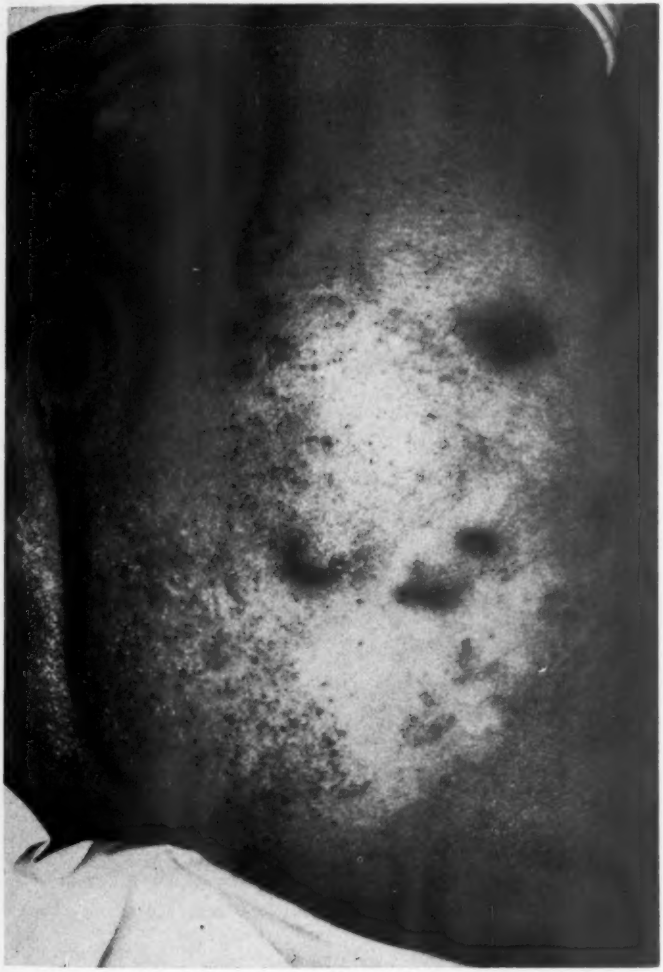

FIG. 3. Pustular *T. rubrum* of the buttocks.

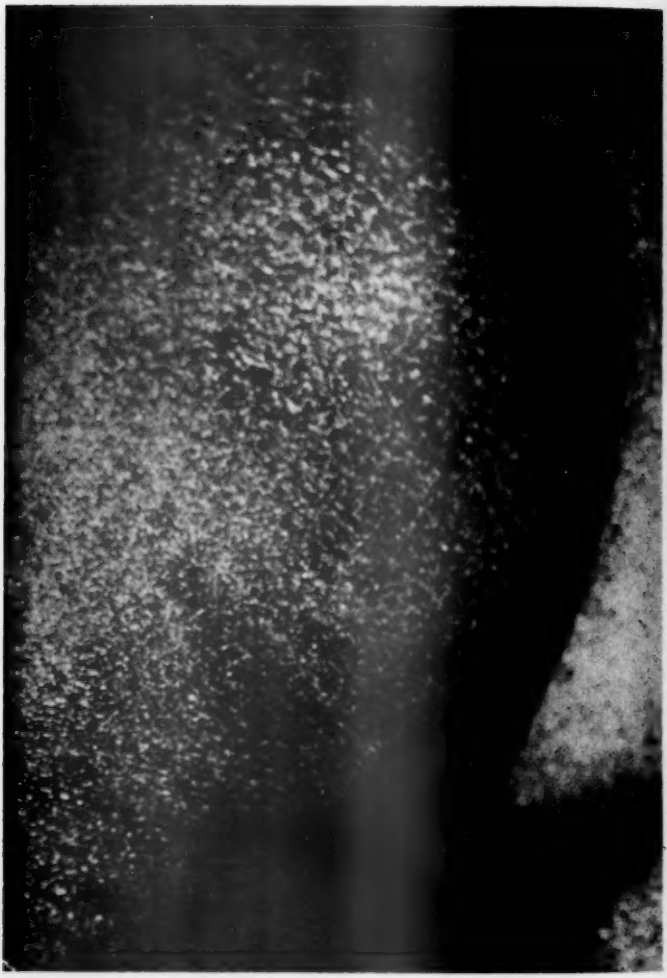

FIG. 4. Patient in Figure 3, six weeks after therapy showing complete clearing.

FIG. 5. Typical *T. rubrum* of the buttocks.

FIG. 6. Closeup of Figure 5 four weeks after therapy showing residual scaling.

Observations for Toxicity

To date, we have been unable to detect any significant changes in hemoglobin levels, liver function tests or urinalyses. During the second week of therapy one patient developed a severe headache which lasted 6 days but which did not necessitate discontinuance of therapy. As mentioned previously, case 1 developed dizziness and when seen by her family physician was found to have an elevated blood pressure. Griseofulvin therapy was discontinued and when the patient was next seen her blood pressure had returned to normal.

The most unusual laboratory changes noted were in the peripheral blood cell studies in 3 cases. In 2 adults, a granulocytopenia developed during therapy with a relative and absolute lymphocytosis. This was in the range of about 25%. In each of these patients, the peripheral blood picture returned to normal after griseofulvin was discontinued. Similar findings were noted in a 2½ year old child on a low dosage of 250 mgms. every third day; however, this child developed a "cold" during therapy and had a granulocyte response which fell again after recovery from this illness. This child also showed an eosinophilia of 5 to 7% which suddenly rose too 19% just prior to discontinuing therapy. During the 10 days we have been following this patient, his abnormal peripheral blood picture has been progressively improving.

Two of our patients had histories of previous penicillin sensitivity, yet neither developed reactions to griseofulvin.

Summary and Conclusions

1. Oral griseofulvin is a valuable therapeutic agent in the management of *T. rubrum* infections, especially in those patients that have failed to respond to topical therapy.

2. In tinea capitis, infected hairs are mechanically replaced by healthy new growth. False positive KOH and Wood's light examinations may persist after cultures are negative.

3. Onychomycosis also heals slowly by mechanical replacement with normal nail.

4. All toxic reactions so far appear to be temporary; however, our series indicates that repeated peripheral blood counts and differentials are required during a course of therapy.

5. Potential relapse to infection exists following griseofulvin therapy.

6. The optimal minimal dosage is yet to be determined.

REFERENCES AND NOTES

1. Blank, H., and F. J. Roth, Jr.: The treatment of dermatomycoses with orally administered griseofulvin. *A.M.A. Arch. Dermat. and Syph.*, 79: 259–266, 1959.

2. Brian, P. W., P. J. Curtis, and H. G. Hemming: Production of griseofulvin by *Penicillium raistrickii*. *Trans. Brit. Mycol. Soc.*, 38: 305–308, 1955.

3. Brian, P. W., J. M. Wright, J. Stubbs, and A. M. Way: Uptake of antibiotic metabolites of soil micro-organisms by plants. *Nature* (London), 167: 347–349, 1951.

4. Gentles, J. C.: Experimental ringworm in guinea pigs: oral treatment with griseofulvin. *Nature* (London), 182: 476–477, 1958.

5. Lauder, I. M., and J. G. O'Sullivan: *Vet. Rec.*, 70: 949, 1958.

6. Oxford, A. E., H. Raistrick, and P. Simonart: Studies in the biochemistry of micro-organisms: LX. Griseofulvum, $C_{17}H_{17}O_6Cl$, a metabolic product of *Penicillium griseofulvum* Dierckx. *Biochem. J.* (London), 33: 240–248, 1939.

7. Pipkin, J. Lewis: personal communication.

8. Williams, D. I., R. H. Marten, and I. Sarkany: Oral treatment of ringworm with griseofulvin. *Lancet* (London), 7058: 1212–1213, 1958.

[a] From the Department of Dermatology (J. Fred Mullins, M.D., Professor and Chairman), University of Texas Medical Branch, Galveston, Texas. Material in the study was supplied by McNeil Laboratories, Inc., a subsidiary of Johnson & Johnson. The product will be marketed as Grifulvin.

EDITORIAL COMMENT

In volume 17 of TEXAS REPORTS we tried the experiment of printing a number of clinical papers which are of teaching value but do not conform to our stated criterion, "critical evaluations of clinical medicine and medical research." We would appreciate comment on this experiment as well as on any feature of the REPORTS which may increase reader interest.

DEPARTMENT OF RADIOLOGY
CASE OF THE WEEK

MELVIN SCHREIBER[a]

FIGURE 1

The patient is a 34 year old white male who complains of blurred vision, nausea, uncontrollable intermittent twitching of the extremities, and shortness of breath. Long history of albuminuria. Hemoglobin 5 grams %.

The patient succumbed shortly after the problem film was exposed. The pertinent autopsy findings included glomerular hya-

FIGURE 2

linization with necrosis, renal arterial and arteriolar thickening, fibrosis of the renal parenchyma, intrapulmonary fluid transudation and hemorrhage, fibrous thickening of the alveoli, and fibrinous pericardial exudate. The final pathological diagnoses included chronic gomerulonephritis with acute exacerbation, arterial and arteriolar nephrosclerosis, pulmonary congestion, edema, and hemorrhage, and fibrinous pericarditis, probably uremic. The NPN four days before death was 336 mg. %.

The characteristic picture of pulmonary edema consists of a diffuse increase in density around the hilar portions of both lungs which fades out toward the periphery. The density is ordinarily less than that of most pneumonic consolidations, and the apices and bases usually remain clear. The terms "butterfly wings" and "bat wings" are often appropriate descriptive phrases. In pulmonary congestion the radiographic appearance is that of enlarged peri-hilar vessels with blurred and indistinct outlines, often accompanied by cardiac enlargement. The densities in pulmonary edema are more extensive and represent the additional feature of alveolar fluid, often without hilar vessel enlargement. Indeed, increase in the size of the pulmonary vessels in the presence of uremic pulmonary edema suggests superimposed left ventricular failure.

The microscopic features of uremic pneumonitis include a moderate amount of protein-rich alveolar edema with an amorphous or granular protein percipitate. A fine fibrin network is often present in the alveoli. A few erythrocytes and varying numbers of macrophages in the alveoli complete the picture.

The pathogenesis of uremic pulmonary edema is unsettled. Increase in capillary permeability due to toxic products associated with azotemia may be responsible for the outpouring of fibrin into the alveolar spaces, but passive congestion incident to some degree of left ventricular failure, related or not to the hypertension which frequently accompanies chronic renal disease, may play a part in the production of this change.

REFERENCES

Hopps, H. C., and Wissler, R. W., Uremic Pneumonitis, *Am. J. Path.*, 31: 261–273, 1955.

Gould, D. M., and Torrance, D. J., Pulmonary Edema, *Am. J. Roent.*, 73: 366–374, 1955.

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SUMMARY DESCRIPTION OF MULTIPLE IMPACT PSYCHOTHERAPY[1]

FRANK P. SCHUSTER, JR.

A new method for brief impact psychotherapy of the family as a unit is being evaluated by the Youth Development Project in Galveston for possible use in outpatient psychiatric clinics.

A great proportion of the referrals to this clinic, from all corners of the state of Texas, require a considerable investment of psychotherapeutic time for proper management. This indicated treatment is generally unavailable without severe dislocation of the family since a majority of the patients live in areas of the state remote from a psychiatric facility which could make possible a continuing therapeutic program.

Taking note of the fact that in our experiences with adolescents the determining changes often occurred early in the course of conventional psychotherapy, and in agreement with the premise that the disturbed child is best helped by treating the disturbance in the whole family, the staff and consultants of the Youth Development Project initiated a pilot study, in 1957, to test the feasibility of an intensive brief psychotherapy method. The results of this pilot study warranted a full scale evaluation, now in operation.

For two consecutive days, from morning till night, adolescent, mother, father, siblings, are engaged in intensive therapy by a four member interdisciplinary team. Various combinations of group, joint, and individual sessions permit, in criss-crossing and overlapping interviews, the rapid development of a dynamic history of the crisis. In group meetings the patterns of family interaction are observed directly and there is an effort to label promptly distorted and unrealistic attitudes. These interpretations are tolerable because of group support—team and family alike.

In each following individual or joint session the genesis of these distortions is examined. Beginning insights are shared in overlapping interviews, and are submitted for validation in group or joint sessions. By the end of the second day there is some real clarification of the main issues confronting the family, and,

if we have been successful, the family's own reconstructive forces have in large measure been mobilized.

We have two criteria for selection of patients: (1) the adolescent must be so disturbed that the community has given serious thought to placing him in a hospital or in a training school; (2) parent figures and significantly involved siblings must agree to participate in the multiple impact procedure along with the nominal patient.

The presenting complaints run the gamut from sexual deviation and markedly antisocial behavior to the "school phobias." Treatment goals are adjusted to the degree of psychopathology encountered. Psychiatric disorders range, in the adolescent and in his family from the behavior problems through chronic schizophrenia.

Our preliminary six-month and one year follow-up study suggest this method is at least as effective as long term individual treatment for many adolescent referrals.

The first day procedures begin with a team family conference. The family demonstrates their gross patterns of interaction by their opening attempts to describe and define the "problem."

The team participates by commenting principally about the roles the family members seem to be playing. The need to repudiate some of the team's observations brings about intense participation by the family.

The team does not strive for "impact" by being assaultive—the material being dealt with is in itself anxiogenic—the approach is always supportive.

Nonetheless, the family's tension builds to a nearly intolerable level and the group meeting must be dissolved into individual or joint interviews by mid-morning.

In the privacy of the individual interview, the family member begins to blurt out all the things he couldn't say with everyone sitting around. This burst of ventilation is encouraged for a time. Then there is a direct inquiry about the significant historical facts underlying these feelings.

Half way through any individual interview, another team member may telephone for permission to join in. The first therapist asking rhetorically for permission "to fill in" the newcomer, summarizes both the factual and affective content of the interview. He may draw conclusions and make interpretations

during this brief recapitulation. The family member is urged to take exception if he disagrees, during the course of this summarization, or to make needed corrections.

The second team member reflects back what he has heard, and again the family member is asked to validate the impressions or to correct them. This redundancy is felt to be important. The second team member then adds information about the factual and affective content of the interview he has just left.

The breach in confidentiality is thus accomplished in a relatively painless fashion. There is tacit acceptance on the part of each family member that his confidence will be breached whenever necessary. Longstanding barriers to communication within the family are broken down directly in this manner, with therapeutic results.

During lunch, apart from the family, the team shares pertinent historical data and marks out obscurities and contradictions for investigation. Positive or negative counter-transferences are examined and may be caricatured to explore their possible usefulness during the afternoon.

On the afternoon of the first day we inquire into the inevitable disparities in the histories as given by the several family members. If the mother, for example, is evasive and protests inexact memory, the therapist may suggest a joint interview with the father or the son, who might be able to clarify our understanding of some significant event in the past. The ready access to verifying sources promotes emotional and intellectual honesty. Conscious evasion and distortion, which so often delays a definition of the real pathologic trends in a family, is thus discouraged. Doubtful issues are hammered out on the spot.

As the personality structure of the parents is clarified, areas of unrealized potential for emotional growth are focused upon. The parents' right to and necessity for healthy self interest is stressed, and the drab preoccupation with the woes and manipulations of their adolescent is decried.

In a team-family conference late in the afternoon of the first day there is evidence of beginning insight as to what the current crisis is about. Problem areas have been identified. Planning about the current crisis, however, except as it reflects more general pathologic patterns within the family, is deferred until the following day.

The family repairs to quarters they have arranged for themselves. Most often there is a "long talk" by the parents—only rarely is the adolescent included. The day's intensive therapy has often fostered a healthy rediscovery by the parents of their interest in each other, providing what may be the first good emotional interchange in years.

Typically, on the second morning, in a team-family conference, there is a report on the evening before. The parents, between whom communication has improved significantly, may offer suggestions for self-correction. Often histoical data recalled during the night is added. The family is congratulated on their new found collaborative ability, but the pace does not slacken. In individual and multiple-therapist sessions the new material is enlarged upon and its significance further explored.

During this process of exploration, the previously deferred questions pertaining to the current crisis are answered, at least, in part, by the formulations the family itself is making. We are now beginning to get feedback. The team participates, if necessary, by offering either suggestive or highly directive formulations of their own.

The family is encouraged to draft during their lunch hour, again apart from the team, a specific course of action for the immediate future.

It is openly predicted that the adolescent will have to test out, and perhaps act out against, the new interpersonal equilibrium in the family.

After lunch, the team becomes less directive, and the family members are drawn out and encouraged to put together their plans for the future. The team participates by rephrasing what it has heard from the family, and may, on occasion, ask for possible alternative solutions to the problems posed by the crisis. When a reasonably definite plan of action has been derived, the group conference is broken up again for individual or joint interviews.

Each therapist encourages the patient to criticize the family plan from his own highly personal viewpoint, permitting him to voice objections which were muted in the team-family conference. These difficulties are pondered, talked over, and alternatives reached for. Then joint conferences between various mem-

bers of the family are arranged, to "iron out" with the therapists' help, the unresolved parts of the family solution.

Joint and individual interviews alternate, with separation and recombination of family members perhaps four or five times during the afternoon. These sessions do not end until a high degree of resolution has been effected. At that point, a team-family conference is called, plans are re-stated, and each family member is asked to verbalize his understanding of the joint team-family formulation.

The family is assured of our continued interest in them, and plans are made for a six-month follow-up evaluation. A minimum of dependency is encouraged. The family is given a vote of confidence and advised to trust their own remobilized resources rather than search outside of the family for help.

On this note, the two-day treatment period comes to an end.

As the project enters its second year, it has become apparent that the implications of this therapeutic approach are of broader scope than initially assumed. The method has potential not only as a tool for investigation into the complex problem of family diagnosis, but also seems adaptable to a wide variety of treatment and training applications. Exploration into these applications and their limitations is currently underway. Reports of these studies, detailed clinical case material and analysis of follow-up data will be presented in subsequent publications.

[1] This demonstration project was supported in its pilot year by a grant from the Hogg Foundation for Mental Health, and is currently supported jointly by the Department of Psychiatry, University of Texas Medical Branch at Galveston, and by the National Institute of Mental Health (Project grant OM 76 N.I.M.H.). The directors of the Youth Development Project are Harold A. Goolishian, Ph.D., and Eugene C. McDanald, M.D. Staff team members are Robert MacGregor, Ph.D., Agnes Ritchie, M.S.W., Cullen Mancuso, M.A., and Frank P. Schuster, Jr., M.D.

PENETRATING ABDOMINAL WOUNDS

A. O. Singleton, Jr., and Jerry D. Julian[a]

Penetrating Abdominal Wounds

This report is an analysis of 152 consecutive penetrating wounds of the abdomen treated by the surgical staff at John Sealy Hospital, Galveston, Texas in a 12 year period from January 1, 1947 through December 31, 1958. There has been no significant change in the management of this type of wound during this period. Twenty cases, in which it was necessary to do an exploratory laparotomy to determine that penetration of the abdominal cavity had not occurred, were not included in this study.

Of the 152 penetrating abdominal wounds operated upon during this period, the lowest annual incidence was in 1950 with four cases, while the highest number occurred in 1958 with 28 cases.

Sex, Race and Age Distribution

Table I gives the sex, race, and age distribution found in this series. Males outnumbered females about six to one, with 131 (86.2%) males and 21 (13.8%) females. Although colored patients averaged only about 32% of all admissions, there were 94 (61.8%) negro to 58 (38.2%) white. A report from this hospital by Moore and Kennedy covering the years 1931–1941 revealed that there was a total of 51 negro (68%) and 24 white (32%) in a series of 75 consecutive penetrating wounds. The racial distribution, therefore, has not changed significantly in this hospital for this type of injury.

The age group of 20 to 40 years accounts for about two-thirds

Table 1

Sex, Race, and Age Distribution in 152 Penetrating Abdominal Wounds

	Sex		Age Groups			Race	
	Male	Female	Under 20	Over 20 Under 40	Over 40	White	Negro
No. Cases	131	21	23	99	30	58	94
Percentage	86.2	13.8	15.1	65.1	19.8	38.2	61.8

of the total number of cases with 99 (65.1%). The age group over 40 years numbered 30 (19.8%) cases, while the age group of less than 20 years accounted for only 23 (15.1%) cases.

Wounds, Incisions, and Blood Used

An analysis of wounding agents, operative incisions, and blood used is indicated in Table II. Stab wounds outnumbered gunshot

TABLE 2

Analysis of Wounds, Incisions and Blood Use in 152 Penetrating Abdominal Wounds

	Wounds			Exploratory Incisions		
	Stab	G.S.W.	Misc.	Trans.	Vert.	Blood Used
No. Cases	97	48	7	141	11	103 (2.75 units per case)
Percentage	63.8	31.6	4.6	92.8	7.2	67.7

wounds two to one, with a total of 97 (63.8%) stab and 48 (31.6%) gunshot wounds. Moore and Kennedy reported 40 (53.3%) stab wounds and 35 (46.7%) gunshot wounds in their series. There was a total of seven miscellaneous wounds in our group where a razor blade (1), broken beer bottle (1), and shrapnel (1) were the causative factors. Also included were the following: a case of small bowel perforation secondary to paracentesis (1), uncontrolled peritoneal hemorrhage secondary to needle liver biopsy (2), and one case in which a Steinmann pin protruded through the acetabulum and penetrated the peritoneal cavity.

The incisions used in exploring the abdominal cavity were predominantly of the transverse type. A total of 141 (92.8%) transverse incisions were used as compared to 11 (7.2%) vertical incisions. There was one wound dehiscence with evisceration in this series (0.65%) occurring in a transverse incision. This complication was in an un-cooperative patient who was out of bed against orders four days postoperative, when he developed an attack of coughing and eviscerated a large portion of small intestine. Hamilton and Duncan reported an incidence of 5.6% in 236 cases, and Walker reported a 7.3% incidence in 95 penetrating abdominal wounds. We believe that transverse incisions

are generally preferable since the direction of the pull of the abdominal muscles, as well as their fibers, is in a transverse plane. This makes closure easier and lessens the chance of hernia and wound disruption. In special situations, a vertical incision may be indicated, although a flank to flank transverse incision allows good exposure everywhere in the abdomen.

A total of 283 blood units (500 cc. per unit) was used for an average of 1.86 blood units per case for all cases. McComb et al., in a recent report from the Robert B. Green Memorial Hospital, San Antonio, Texas covering a six year period involving 311 penetrating abdominal wounds, used an average of 2.83 blood units per case. In our series, blood transfusions were used in 103 (67.7%) cases. These 103 cases received an average of 2.75 blood units per case.

The anesthesia used in all cases except one was general intratracheal anesthesia. One spinal anesthetic was given.

The average hospital stay was 12.8 days.

In general, penicillin and streptomycin were used prophylactically. When there was evidence of infection, various broad spectrum antibiotics were also used.

Visceral Injury

Hollow visceral, solid visceral, and non-visceral wounds are illustrated in Table III. The small bowel (including the duodenum, jejunum,, and ileum) was injured in 52 (34.2%) cases.

TABLE 3

Wounds of Hollow Viscera, Solid Viscera, Diaphragm and Peritoneum Only in 152 Penetrating Abdominal Wounds

Hollow Viscera			Solid Viscera		
Organ	Total	Per Cent	Organ	Total	Per Cent
Small Bowel	52	34.2	Liver	49	32.2
Colon	35	23.0	Kidney	9	5.9
Stomach	21	13.8	Spleen	5	3.3
Vascular	9	5.9	Pancreas	3	2.0
Urinary Bladder	6	3.9	Peritoneum only, diaphragm, mesentery		
Rectum	5	3.3			
Gallbladder	2	1.3	Peritoneum only	28	18.4
Appendix	1	0.65	Mesentery	21	13.8
Ureter	1	0.65	Diaphragm	20	13.2

Primary simple closure was used in these cases unless there were numerous perforations in a localized area, or the vascular supply to an area was obviously impaired, in which case a resection with end to end anastomosis was generally used.

The colon was the next most frequently injured hollow viscus with a total of 35 (23.0%) injuries. Of these injuries, 32 had perforation into the lumen, while the remainder were injuries which did not enter into the lumen. Of the perforating injuries only four were treated by colostomy, while 28 were treated by primary closure. One of the cases of colostomy developed a prolapse six weeks after surgery. This was resected and bowel continuity re-established. Neomycin irrigation of the peritoneal cavity was used in ten cases with gross fecal contamination from perforations into the colon, with five of these cases also receiving neomycin injected into the bowel both proximal and distal to the injured segment. In five other cases neomycin was injected into the bowel only. There were no cases of peritonitis in the 15 cases of colon perforation in which neomycin irrigation was used. However, only one case of recognizable postoperative peritonitis occurred in the entire series. This appeared in a patient with a colostomy performed for a perforation of the colon at the splenic flexure. In 19 other colon perforations where no irrigations were used, no peritonitis occurred.

The stomach was injured 21 times (13.8%). Primary closure was used as the method of choice for repair in all cases.

Table IV gives further data on gastrointestinal wounds.

There were nine (5.9%) significant vascular injuries which included perforation of the vena cava (2), renal vein (1), abdominal aorta (2), gastroepiploic artery (1), superior mesentery artery (1), common iliac vein (1), and hypogastric artery (1). In both cases of abdominal aorta injury the patients succumbed from shock and hemorrhage. One patient with perforation of the inferior vena cava expired at the end of surgery from aspiration of gastric contents. Blood loss was not a factor in this death. One patient died from loss of blood from lacerations of numerous large mesenteric vessels, including the superior mesenteric artery. Another patient expired from shock and hemorrhage due to injury of the common iliac vein and the hypogastric artery.

The urinary bladder was injured five times (3.3%). Only two injuries penetrated through the mucosa, one being closed pri-

TABLE 4

Perforations of the Gastrointestinal Tract in 152 Penetrating Abdominal Wounds

Organs Perforated	No. Cases	Type of Injury			Died
		Misc.	Stab	G.S.W.	
No gastrointestinal perforation	84	4	71	9	4
Stomach	2	0	0	2	0
Colon					
Small bowel					
Stomach					
Small bowel	1	0	0	1	0
Stomach					
Colon	3	1	1	1	0
Colon					
Small bowel	19	0	1	18	2
Stomach	12	0	7	5	1
Small bowel	23	2	13	8	1
Colon	8	0	4	4	1

marily and the other closed from the inside with a suprapubic cystostomy.

The rectum was injured five times (3.3%). Primary closure was done in one case, and treated by proximal colostomy in four others with good results.

The gallbladder was injured two times (1.3%). In only one case was there perforation through the mucosa. Both injuries were closed primarily.

The appendix was injured only once (0.6%).

Only one ureteral injury occurred in this series and was found at autopsy in a patient with a gunshot wound who expired during surgery of shock and hemorrhage.

An analysis of the solid-viscera injuries shows that the liver was injured 49 times (32.2%). In many of these, bleeding had ceased by the time of the operation. In larger lacerations, the method used for closure was deep sutures over a gelfoam bolster. The liver injuries were drained to prevent bile peritonitis.

The kidneys were injured in nine (5.9%) cases. In only two cases was nephrectomy necessary.

The spleen was injured five times (3.3%), necessitating splenectomy. One death occurred in a patient with injury of the spleen. However, this was not a contributing cause as the patient died eight days postoperative from general peritonitis secondary to a colon injury.

The pancreas was involved in three (2.0%) cases. These were drained to prevent collections of leaking pancreatic juice in the peritoneal cavity.

The mesentery of the small and large bowel was injured significantly in 21 (13.8%) cases. The careful ligation of bleeding vessels and closure of openings in the mesentery to prevent internal herniation has been the treatment of choice.

There were 20 chest wounds which resulted in penetration into the peritoneal cavity, which indicates the thorough abdominal examination necessary in all patients with chest injuries in which the actual path of the wounding agent can not be determined. Abdominal injury must be suspected in all wounds of the lower chest.

There was a total of 28 (18.4%) cases of perforation into the peritoneal cavity without evidence of visceral injury being found by exploration of the abdomen. A more detailed discussion of the treatment of abdominal injuries has been given in a previous publication (4).

Complications and Deaths

The complications which occurred in this series of 152 cases are found in Table V. These have been divided into major and minor complications on the basis of whether the complication was a significant threat to the patient's life or significantly increased the hospital stay. There were 15 (9.8%) major and 28 (18.4%) minor complications with an overall rate of approximately 28.3%. (Although not serious here, the "minor complications" listed have been major problems at times in postoperative cases.)

An analysis of deaths in relationship to time lapse from injury until operation was attempted. The average time was 5¾ hours. Since so few cases were seen late, it is difficult to draw any conclusions in relation to this factor.

The over-all death rate in this series of 152 cases was 5.9% with nine deaths. There were three deaths from 97 stab wounds

TABLE 5

Complications of 152 Penetrating Abdominal Wounds

Major			Minor		
Complication	Number	Per Cent	Complication	Number	Per Cent
Intest. Obstruction	6	3.9	Wound infection	20	13.2
Subphrenic abscess	3	2.0	Thrombophlebitis	3	2.0
Peritonitis	2	1.3	Hematoma of Wound	2	1.3
Evisceration	1	0.65	Adynamic ileus	1	0.65
Prolapse of colostomy	1	0.65	Pneumonitis	1	0.65
Gangrene-Abd. Wall	1	0.65	Drug reaction	1	0.65
Grand mal seizure	1	0.65			
Totals	15	9.87		28	18.4

for 3.09% mortality, and six deaths from 48 gunshot wounds for a 12.5% mortality. There were no deaths in the miscellaneous group. Six patients died at operation or in the immediate postoperative period, five from shock and hemorrhage, one from massive aspiration of gastric contents at time of extubation. One died from unknown causes following convulsions with decerebrate rigidity on the fourth postoperative day. One died of peritonitis on the eighth postoperative day. One patient eviscerated on the fourth postoperative day and died of localized peritonitis and bilateral bronchopneumonia three days later.

McComb et al., reporting on 311 penetrating abdominal wounds (1950 to 1956) had six deaths from 243 stab wounds for a 2.5% mortality, and 14 deaths from 64 gunshot wounds for a 21.8% mortality. The total operative mortality rate in this series was 6.4%.

Moore and Kennedy, in a report from this hospital (1931–1941) reported 40 stab wounds with four deaths for a 10% mortality, and 35 gunshot wounds with 13 deaths for a 37.1% mortality. The average mortality for this series of 75 cases was 22.6%. Hamilton and Duncan, reporting on 236 cases of penetrating abdominal wounds from 1930 to 1941, had 146 stab wounds with 14.1% mortality, and 190 gunshot wounds for a 51% mortality, giving a total mortality of 31.1%.

The significant changes in the therapy for patients with penetrating abdominal wounds, comparing the period of 1930–40 and that of 1947–58, have been the extensive use of whole blood, plasma, and plasma expanders, and the use of antibiotics. These

two stalwarts of modern therapy would seem to be mainly responsible for the reduction in mortality rates in penetrating abdominal wounds.

Summary

A total of 152 penetrating abdominal wounds were treated at John Sealy Hospital, Galveston, Texas from January 1, 1947 through December 31, 1958. Males outnumbered females six to one, and with a ratio of negroes to whites of two to one. Most of the cases were in the 20–40 year age group. Stab wounds overshadowed gunshot wounds two to one.

The over-all mortality rate of the series was 5.9%, with a higher percentage of deaths from gunshot than stab wounds. This represents an improvement over the previous mortality figure of 22.6% reported from our institution covering the period, 1931–1941.

REFERENCES AND NOTES

1. Hamilton, J.E., and Duncan, E.: Penetrating gunshot and stab wounds of the abdomen. *Surgery* 13: 107–121, 1943.
2. McComb, A. R., Pridgen, J. E., Hills, W. J., Smith, R., Gregory, E. E., Sammis, W., Wright, R. R., and Herff, A., Jr.: Penetrating wounds of the abdomen. *Am. Surgeon* 24: 123–131, 1958.
3. Moore, R. M., and Kennedy, J. C.: Mortality in penetrating wounds of the abdomen in civil practice with particular reference to the influence of hemorrhage. *War. Med.*, 2: 912–916, 1942.
4. Moore, R. M., and Singleton, A. O., Jr.: Penetrating Wounds of the Abdomen. *Am. J. Gastroenterol.* (In press)
5. Walker, W. H.: Penetrating abdominal wounds. *Arch. Surg.*, 60: 55–64, 1950.

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LOWER RIO GRANDE VALLEY OUTBREAK OF ST. LOUIS ENCEPHALITIS[a]

THELMA D. SULLIVAN[b], JAMES E. GRIMES[c], J. V. IRONS[d], AND J. E. PEAVY[e]

Some of the laboratory aspects of an outbreak of 120 cases of St. Louis encephalitis (SLE) primarily in Cameron County Texas the summer of 1957 is the subject of this report. The attack rate was slightly in excess of 150 per 100,000 inhabitants. Most of these cases occurred in July and early in August, near Harlingen. Some of the cases occurred in personnel of the Harlingen Air Force Base. An even larger outbreak had occurred primarily in the adjoining Hidalgo County in 1954.[1] Paired blood samples from cases of encephalitis were obtained for complement fixation tests. Pooled mosquitoes of several species from the affected area also were tested for virus content.

Methods and Materials

A quantitative complement-fixation test[1] was performed on serums from patients. All serum samples from the same patient were tested simultaneously when it was possible. Titers were based on the last tube showing 3+ or 4+ fixation. In addition to use of St. Louis (SLE) antigen, Western equine encephalitis (WEE) and Eastern equine encephalitis (EEE) antigens were employed. With cases showing poor or no serologic response to St. Louis antigen, lymphocytic choriomeningitis (LCM) and mumps antigens were employed. The serum virus neutralization test[2] was not done.

Several paired serums were screened with influenza or other antigens in HI or other tests.

An agent was sought in a few selected throat swabs, spinal fluids, serums and stools by the inoculation of monkey kidney (MK) or HeLa cell cultures and mice.

A possible animal or bird reservoir was not investigated. Relatively few horses were in the area affected and no cases of equine encephalomyelitis had been reported. The area has a large bird population, including many chickens and other domestic birds. A heavy population of many species of wild or migrating birds is found in this semi-tropical area. After a rainy period, dry weather favored the propagation of numerous *Culex quinquefasciatus* mosquitoes which were found in many places. Several species of mosquitoes, few of which are known vectors of encephalitis, were collected by an entomological team and brought or sent to the laboratory. Several pools of mosquitoes were identified and processed by accepted procedures for possible virus content.

Results

In complement-fixation (CF) tests, four-fold or greater SLE titer rises were demonstrated in 14 cases representing 42.4% of the 33 clinical encephalitis cases tested. Paired serum samples from 9 cases failed to show CF titers at least at the 1:4(3+) level. Ten cases showed no significant change in titer, although 5 were read at 1:32(3+) or higher. The extent of change in titer of serums obtained from 33 patients is shown in Table I. The need for collection of an early blood specimen preferably by beginning of second week after onset of illness is evident. Titers tended to fall after several weeks.

Two of 4 single convalescent serums showed SLE titers at 1:32(3+) or higher.

Paired serum samples from the 33 clinically diagnosed cases of encephalitis showed negative CF tests in which WEE, EEE, and LCM antigens were used. A four-fold rise in titer with mumps antigen was demonstrated in one instance.

Paired serum samples from serologically non-reactive clinical

TABLE 1

Complement Fixation Antibody Pattern of 31 Serums

Base line serum taken (days)	Results	Followup serum taken (days)					
		0–7	8–14	15–21	22–36	37–85	Later
0–7	Negative	2	.	.	.	.	.
	No change	.	2	.	2	.	.
	Rise	1	4	2	1	.	.
	Decline	.	.	.	.	.	.
	Total	3	6	2	3	0	.
8–14	Negative	.	.	.	.	.	.
	No change	1	2	2	.	1	.
	Rise	.	1	1	2	.	.
	Decline	.	.	.	.	.	1
	Total	1	3	3	2	1	1
15–36	Negative	.	.	.	.	.	.
	No change	.	1	1	1	1	.
	Rise	.	.	1	.	.	.
	Decline	.	.	.	.	.	1
	Total	.	1	2	1	1	1

Note: No change means antibodies present, but without significant fourfold rise.

cases of encephalitis also showed negative hemagglutination-inhibition tests for "Asian flu." "Asian flu" cases began to occur at the end of the outbreak of encephalitis and became quite prevalent in October.

Of the serums obtained from 426 persons who presumably were exposed but were not ill, 29.8% had SLE titers at 1:4(3+) levels or higher. Most of these specimens were collected near the peak of the outbreak.

Two early acute phase serum samples, 2 throat swabs, 2 spinal fluids and 5 stool specimens failed to yield an agent by inoculation of mice or tissue cultures.

In August, a total of 1900 mosquitoes, 85% of which were *Culex quinquefasciatus*, were collected and later tested for virus content. Eight of 40 pools of *Culex quinquefasciatus* showed presence of SLE virus. These eight pools contained from 20 to 100 mosquitoes each. Most of the collections were made near Harlingen.

These eight agents in tests in mice were neutralized by stock SLE antiserum. WEE and other antiserums failed to show neutralizing activity against these agents. One of the eight strains was utilized for immunization of a group of mice. Pooled antiserums from the survivors neutralized this and the other strains as well as a stock SLE virus but failed to neutralize WEE or other viruses tested. A freshly isolated agent showed a viremia in young chicks, and this agent was identified as SLE virus.

Discussion

This study again proved the value of the CF test in the investigation of an outbreak of encephalitis. By means of the CF test, the identification of several illnesses firmly established this as another outbreak of SLE. The success in obtaining useful serologic data was primarily due to the availability of multiple specimens. Paired specimens properly spaced permitted detection of a significant four-fold or greater titer increase in a good percentage of cases. When the first specimen was obtained during the early acute illness, the second of the pair of blood specimens could be taken several days or a few weeks later. In some instances, the first blood specimen was taken too late to show a significant titer rise. In some other cases both blood specimens were taken too soon or the interval was too short to permit a significant rise in

titer. In a few cases it appeared that appreciable CF antibodies failed to develop.

The frequent finding of CF antibodies in serum samples from persons who presumably were exposed but who were not ill suggested that the SLE virus can cause many inapparent infections, in line with the findings in 1954.

Little emphasis was placed upon the direct identification of the SLE virus in specimens from the patient, since this is not a productive procedure.[2]

Successful repeated findings of SLE virus in small pools of *Culex quinquefasciatus* mosquitoes emphasized the probable importance of this mosquito in the dissemination of the SLE virus and progress of the outbreak. *Culex tarsalis* which is so firmly established as the important vector of SLE farther west[3] and of WEE in many areas[4] was not found in significant numbers. The occurrence of WEE, EEE, and SLE viruses in the Lower Rio Grande Valley had been previously recognized.[5] Both in the 1954 and the 1957 outbreaks there was no evidence of the presence either of the WEE or EEE viruses.

Summary

The complement-fixation (CF) test was found to be of great value in detecting significant titer increases when blood specimens were properly spaced.

By means of the complement-fixation test, the Cameron County outbreak of encephalitis in 1957 was shown to be caused by the St. Louis encephalitis (SLE) virus.

Inapparent infections occurred frequently on the basis of CF titers on persons not ill.

The repeated finding of SLE virus in pools of *Culex quinquefasciatus* supported the identification of the outbreak and indicated the importance of this mosquito as a vector of SLE.

REFERENCES

1. Sullivan, T. D., J. V. Irons, and M. M. Sigel: An outbreak of St. Louis Encephalitis in the Lower Rio Grande Valley of Texas: Laboratory Aspects. *Pub. Health Rep.* 72: 526–530, June, 1957.
2. Hammon, W. McD.: Encephalitis. In Diagnostic procedures for virus and rickettsial diseases. New York, N.Y., American Pub. Health Association, pp. 169–206, 1956.
3. Reeves, W. C.: The knowns and the unknowns in the natural history of encephalitis. *Proc. and Papers, 21st Annual Conference of the California Mosquito Control Association*, 1953.

4. Harmston, F. C.; Schultz, G. R.; Eads, R. B., and Menzies, G. C.: Mosquitoes and Encephalitis in the Irrigated High Plains of Texas, *Pub. Health Rep.*, 71: 759–766, Aug. 1956.

5. Hammon, W. McD.; Reeves, W. C.; and Irons, J. V.: Survey of the arthropod-borne virus encephalitides in Texas with particular reference to the Lower Rio Grande Valley in 1942. *Texas Rept. Biol. & Med.* 2: 366–375, Winter, 1944.

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EDUARDO BRAUN-MENÉNDEZ

(1903–1959)

With the passing of Eduardo Braun-Menéndez, who was killed in an aeroplane accident on his birthday, the world of science lost one of its most distinguished representatives. Born in Argentina and trained at the universities of Buenos Aires, Paris, Vienna and London, he joined the staff of Professor Bernardo A. Houssay, whose chair of physiology he accepted in 1955. His important investigations on heart sounds, on hypertension, compensatory hypertrophy of the kidney, metabolism of the heart muscle, etc. brought him an international reputation. However, Braun-Menéndez' interest went far beyond pure research. He was extremely active in organizing science in his country and abroad. In 1944 he organized, with Bernardo A. Houssay, the Sociedad Argentina para el Progreso de las Ciencias, established "Ciencia e Investigacion" a popular scientific journal on truly high standards. He was co-founder of a number of very important scientific journals, like "Revista Argentina de Cardiologia," "Acta Physiologica Latinoamericana" and of "Medicina." Recently Braun-Menéndez was active in the organization of the Society for Medical Ethics, which was the first step in the formation of an international society dedicated to these problems. He was interested in the history of medicine and science, and jointly with Luis F. Leloir edited in Spanish a series of classics in this field. He also was active in the establishment of a Catholic University in Argentina.

His scientific work brought him many honors: he had a D.Sc. degree "honoris causa" from the University of California and an honorary M.D. degree from the University of Brazil. He was honorary member of many scientific societies, among others American College of Physicians, New York Academy of Medicine, Societé Française de Cardiologie, Societé de la Pathologie Rènal de Paris, Academy of Sciences of Brazil, etc. He also was associate member of the Physiological Society of London, and Schweizerischer Verein der Physiologen und Pharmakologen. The Medical Schools of the Western Reserve University, Uni-

versity of Chicago, University of California, and others invited him to give Memorial Lectures.

Braun-Menéndez maintained particularly close ties with the University of Texas Medical School: he was an honorary lecturer in physiology and in this capacity visited Galveston on several occasions, giving lectures and conducting discussions with members of the Faculty. All of those who had the privilege of knowing him were impressed by his great kindness, warm personality, his extensive knowledge of Biology and Medicine and his deep philosophical background. A perfect gentleman and a person of highest culture, Braun-Menéndez could say with Terrence "homo sum et nihil humani me alenium esse puto." He is mourned not only by his family.

Wiktor W. Nowinski

CALLING ATTENTION TO

April, 1959

Wow! Atlantic City session of Federation of American Societies for Experimental Biology April 13–17 offers 2383 abstracts (with author index of 30pp), joint session April 14 on biology of fine structure of macromolecules (FHC Crick Chairman), & 38 intersociety sessions on atherosclerosis, blood clotting, cytology, drug action, neurochemistry & neuropharmacology, radiation & tissue culture (*Fed Proc* 18/1–738/59). H Hoagland chairs symposium on fertility-controlling steroids April 14. *Proc* weigh 7.2 Kg.

Virile Virology: E Berger & JL Melnick edit 1st vol of *Progress in Virology* (Hafner,NY, 59, 304 pp, $11.5). C Hallauer & KF Meyer edit *Handbuch der Virusforschung*, Bd 4, III Ergangzungsband (Springer, Wien, 58, 688 pp, DM 96) including FM Burnet on genetics of animal viruses, P Fredericq on genetics of bacteriophages, WC Reeves on arthropods as vectors of pathogenic viruses, & S Bern on polio virus. M Pollard well edits *Perspectives in Virology*, dedicated to FR Beaudette (1897–1957) who edited Prior Hartz Mountain Products Symposia (John Wiley, NY, 59, 331 pp, $10).

Clinical: HK Beecher discusses *Experimentation in Man* (CC Thomas, Springfield, Ill., 59, 80 pp, $2). HR Beck & Co give *Leitfaden des Strahlenschutzes*, helpful for scientists, technicians & physicians (O Thieme, Stuttgart, 59, 253 pp DM 36). S Ebashi & Co to show high creatine phosphokinase activity of sera in progressive muscular dystrophy (*J Biochem* 46/103/59). WT Foulk & Co describe constitutional hypatic dysfunction, or hyperbilirubinemia (*Medicine* 38/25/59). HJ Gross & J Norton note chlorpromazine increases susceptibility to salmonella infections (*Science* 129/784/Mch 20/59). HG Kobrack edits *The Middle Ear* (Univ Chicago Press, Chicago 37, 59, 270 pp, $15). BP Sonnenblick edits important *Protection in Diagnostic Radiology* (Rutgers Univ Press, New Brunswick, NJ, 59, 312 pp, $7.5). J. Weibel describes neuromuscular functional index for prognosis of paralysis (*Dis Nerv Syst* 20/103/59).

General: OE Anderson promotes *The Health of a Nation: Harvey W Wiley's Fight for Pure Food* (Univ Chicago Press, 58, 333 pp, $6). SA Barnett edits *A Century of Darwin* (Wm Heinemann, London, 59, 393 pp, 30 s). M Langsdorf & CS Smith introduce fine discussion on science & art (*Bull Atom Sci* 15/50–93/59). M Mead nicely edits *Writings of Ruth Benedict (1887–1948): An Anthropologist at Work* (Houghton-Mifflin, Boston, 59, 583 pp, $6).

Preclinical: HH Beckett & A Burger edit new *Journal of Medical & Pharmaceutical Chemistry*, Vol 1, 1959, $15. CS Elton surveys *Ecology of Invasions by Animals & Plants* (John Wiley, NY 16, 59, 181 pp, $5.5). JW Fairbairn edits *Pharmacology of Plant Phenolics* (Academic Press, NY 3, 59, 160 pp, $6). HS Goldberg edits *Antibiotics: Their Chemistry & Non-Medical Uses* (Van Nostrand, Princeton, NJ, 59, 675 pp, $15). F Gunner discusses time factor in living growth (*Experientia* 15/1/59). AV Hill reviews delightfully & philosophically the study of heat production in nerve & muscle from 1848

to 1914, as 1st chapter of a coming monograph (*Annu Rev Physi* 21/1/59). G Jasmin & P Bois find methyl-phenidate increases muscle performance & antagonizes muscle depression from tranquilizers (*Canad J Biochem Biophys* 37/417/59). RR Kohn & E Rollerson show relation between loss of swelling of heart muscle proteins and decreased heart contractibility with age (*Proc Soc Exp Biol Med* 100/253/59). JL Koppel & Co show sulhydryl inhibitors increase bloodplatelet breakdown (*ibid* p 229). WW Nowinski well edits *Fundamental Aspects of Normal & Malignant Growth* (Van Nostrand, Princeton, NJ, 59, 900 pp, $22.5). H Peeters edits *Protides of the Biological Fluids* (Van Nostrand, Princeton, NJ, 59, 360 pp, $8.5). W Penfield & L Roberts analyse *Speech & Brain Mechanisms*, the Vanuxem Lectures at Princeton (Princeton Univ Press, Princeton, NJ, 59, 252 pp, $5). JC Skou shows activity of surface-spread enzymes varies with degree of unfolding — spread of side-chains (*Biochim Biophys Acta* 31/1/59). PK Smith gives critical review of *Acetophenetidin* (Interscience Publ, NY, 59, 190 pp, $5.75). FH Stodola analyses *Chemical Transformation by Microorganisms* (John Wiley, NY 16, 59, 134 pp, $4.25). J Turkevich surveys the world of fine particles (*Amer Sci* 47/97/59). PG Weil well reviews *The Plasma Proteins: Their Clinical Significance* (JB Lippincott, Philadelphia, 59, 133 pp, $3). RE Zirkle edits *Molecular Biology* (Univ Chicago Press, Chicago 37, 59, 360 pp, $7.5).

More General: EN Andrade tells about Christian Doppler (1805–1853) of the Effect (*Endeavor* 18/14/59). R Coulborn goes into *The Origin of Civilized Societies* (Princeton Univ Press, 59, 204 pp $4). RH Dicke reminds us of the enigma of gravitation (*Amer Sci* 47/25/59). JC O'Flaherty takes a humanistic view of Max Planck, 1858–1947 (*ibid* p 68). CJ Wiggers enjoys *Reminiscences & Adventures in Circulation Research* (Grune & Stratton, NY 16, 59, 416 pp, $10).

MAY, 1959

Clinical: MD Anderson Hospital staff, Houston, edits *Radiation Biology & Cancer* (Univ Texas Press, Austin, 59, 500pp, $8.5). JE Baer & Co describe saluretic action of hydrochlorothiazide (*Proc Soc Exp Biol Med* 100/442/59). GF Cahill & Co review blood glucose & liver (*Am J Med* 26/264/59). HN Christensen offers *Diagnostic Biochemistry* (Oxford Univ Press, New York 16, 59, 393pp, $6.5). H Linderholm notes diffusing capacity of lungs as limiting factor for physical work (*Acta Med Scand* 163/61/59). G Pincus well edits *Hormones & Atherosclerosis* (Academic Press, New York 3 59, 484pp, $13.5). RK Richards discusses use of analeptics in barbiturate poisoning (*Neurology* 9/228/59). B Stemberg & Co find several hematopoietic regulatory factors in human plasma, some increasing maturation, others decreasing it (*Acta Haematol* 21/78/59).

The Psoralens: AC Curtis introduces important symposium on the psoralens (methoxsalen & other furocoumarins) & radiant energy (*J Invest Dermatol* 2/132–387/59). Discussion includes considerations of radiant energy, radiant energy for medical use, skin pigmentation, chemistry & pharmacology of psoralens, & clinical applications.

Preclinical: MD Bogdonoff & Co show emotional arousal increases serum non-esterified fatty acid levels (*Proc Soc Exp Biol Med* 100/503/59). EJM

Campbell reviews *The Respiratory Muscles & the Mechanics of Breathing* (Yearbook Publ, Chicago, 59, 220pp, $4). KAC Elliott & HH Jasper survey gamma-aminobutyric acid & its nervous functions (*Physiol Rev* (39/383/59). EB Flink & RP Doe show week's lag in synchronizing diurnal adrenal function after air travel time displacement (*Proc Soc Exp Biol Med* 100/498/59). SJ Folley introduces discussion on physiology & biochemistry of lactation (*Proc Roy Soc B* 149/301–424/58). N Freinkel & Co find high myo-inositol in thyroid (*Proc Soc Exp Biol Med* 100/549/59). AB Hertzman surveys masomotor regulation of cutaneous circulation *Physiol Rev* 39/280/59). CH Keene edits symposium on viruses (*Kaiser Edtion Med Bull* 7/1–60/59). NA Lassen discusses human cerebral blood flow & oxygen use (*Physiol Rev* 39/183/59). J LeBlanc shows cyanide reduces chloropromazine hypothermia & disruption of mast cells (*Proc Soc Exp Biol Med* 100/635/59). AL Lehinger & Co show swelling of mitochondria by thyroxine & glutathione (*J Biophys Biochem Cytol* 5/97/109/59). WH Marshall reviews Leao's "spreading cortical depression" with KCI (*Physiol Rev* 39/239/59). CC Pfeiffer & Co show hallucinatory effects of acetylcholine inhibitors (*Neurol* 9/249/59). CP Rhoads takes broad look at genetic chemistry & cancer chemotherapy (*Persp Biol Med* 2/318/59). S Sherry & Co analyse fibrinolysis phenomena (*Physiol Rev* 39/343/59). I Sperba discusses organic ion secretion in forming urine & bile (*Pharmacol Rev* 11/109/59). L Sokoloff analyses action of drugs on cerebral circulation (*ibid* p 1). Our RW Stow & JF Schieve measure minute vol blood flow in specific human tissues, find 1.3 to 82×10^{-4} gm blood per gm tissue per second (*J Appl Physiol* 14/215/59). T & O Wieland describe chemistry & toxicology of amanita mushroom poisons (*Pharmacol Rev* 11/87/59). CG Wilber show inverse relation between swimming capacity & rectal temp, blood lactate, load & aggregation (*J Appl Physiol* 14/199/59). DW Wooley reviews antimetabolites (*Science* 129/615/Mch 6/59).

General: M Bechner outlines concept formation in *The Biological Way of Thought* (Columbia Univ Press, NY, 59, 200pp, $6). HK Beecher describes *Measurement of Subjective Responses: Quantitative Effects of Drugs* (Oxford Univ Press, NY 16, 59, 500pp, $13). PF Clark gives appreciation of Hideyo Noguchi 1876–1928 (*Bull Hist Med* 33/1/59). J Dunner interprets *Baruch Spinoza & Western Democracy* (Philosophical Libr, NY, 59, $3). P Frankl surveys *The Gothic* as a study in history of ideas (Princeton Univ Press, 59, 928pp, $15). MD Grmek reviews ancient slavic medicine (*J Hist Med* (14/18/59). NR Hansen explores *Patterns of Discovery* (Cambridge Univ Press, NY 22, 59, 219pp, $5.5). A Larsen clarifies *What We Are For* (Harper, NY, 59, 173pp, $3). DL Miller divorces *Modern Science & Human Freedom* (Univ Texas Press, Austin, 59, 350pp, $6). W Pagel approaches philosophical medicine through *Paracelsus* (S Karger, Basel, 58, 380pp, Fr70). JH Peck, wise old Utah GP, tells wittily *All About Men* (Prentice-Hall, Englewood, NJ, 59, 260pp, $4). J Pick describes discovery of carotid body (*J Hist Med* 14/61/59). KP Popper outlines *The Logic of Scientific Discovery* (Basic Books, NY 3, 59, 420pp, $7.5). SX Radbill appreciates Robley Dunglison, 1798–1869, Medical Educator (*J Med Educ* 34/84/59). DW Richards & AP Fishman go into

The Fabric of Cardiovascular Concepts: Essays on the Development of Ideas (Oxford Univ Press, NY, 59, 450pp, $12.5). GK Tallmadge surveys early French surgery (*J Internat Coll Surg* 31/225/59). GH Whipple reminisces cheerily (*Persp Biol Med* 2/253/59).